UNIVERSITY OF
ILLINOIS LIBRARY
AT URBANA-CHAMPAIGN
[redacted]

NOTICE: **Return or renew all Library Materials! The *Minimum* Fee for each Lost Book is $50.00.**

The person charging this material is responsible for its return to the library from which it was withdrawn on or before the **Latest Date** stamped below.

Theft, mutilation, and underlining of books are reasons for disciplinary action and may result in dismissal from the University.
To renew call Telephone Center, 333-8400

UNIVERSITY OF ILLINOIS LIBRARY AT URBANA-CHAMPAIGN

[redacted] LIBRARY

L161—O-1096

Q.547
Il6s
1994-1995:1

801-56

ORGANIC SEMINAR ABSTRACTS
1994-95, SEMESTER I

University of Illinois

Department of Chemistry
Box 68 Roger Adams Laboratory
600 S. Mathews
Urbana, Illinois 61801-3731

January, 1995

Copyright © by The Board of Trustees of the University of Illinois

SEMINAR TOPICS

Semester I, 1994-1995

Page

Recent Advances in Biaryl Synthesis by Direct Aryl-Aryl Bond Formation...1
Xiping Su

Site-Selective Hydrolytic Cleavage of Peptides with Transition Metal Complexes........ 9
Eileen Thiessen

Chiral Acyl Nitroso Compounds in Asymmetric Synthesis.................... 17
David Gordon

Recent Advances in Organocuprate Chemistry: Structure and Mechanism of Conjugate Addition........25
Robert Stavenger

Preparation, Stereoselective Transformations, and Application of 6-Hydroxy-3-Pyranones in the Synthesis of Polyoxygenated Compounds................. 33
Bryan Duffy

Polymerizations in Supercritical Carbon Dioxide........41
Keith Hirsch

Antisense Oligonucleotide Analogs as Potential Therapeutic Agents: Synthesis and Properties........49
Heather Sings

The Zaragozic Acids: A New Class of Polyketide Derived Squalene Synthase Inhibitors........57
Alexandra Katauskas

Determination of Reaction Pathway Coordinates by Structure Correlation Analysis of Crystal Structures........65
Peng Zhang

Developments in the Osmium Tetroxide Catalyzed Asymmetric Dihydroxylation of Olefins........73
James Nelson

Digitized by the Internet Archive
in 2012 with funding from
University of Illinois Urbana-Champaign

http://archive.org/details/organicsemi199497univ

RECENT ADVANCES IN BIARYL SYNTHESIS BY DIRECT ARYL-ARYL BOND FORMATION

Reported by Xiping Su September 1, 1994

INTRODUCTION

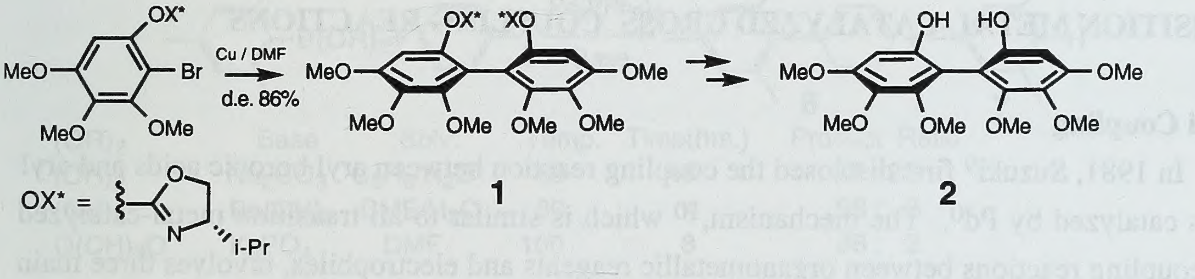
The synthesis of biaryl compounds[1] remains a very active and challenging area in synthetic organic chemistry, not only because many biologically important natural products contain this substructure, but also because biaryls are very important as chiral ligands or chiral auxiliaries for asymmetric synthesis. Biaryls are also useful as conducting polymers, chiral host molecules, chiral phases for chromatography, and chiral liquid crystals.[1a,2]

New methods, mechanistic studies, and especially new atrop-enantioselective reactions have appeared. This review will emphasize recent progress in this area.

THE ULLMANN COUPLING REACTION

The Ullmann coupling reaction[3] is one of the oldest methods for biaryl synthesis. It works best with aryl iodides having chelating substituents in the ortho position. Usually the classical Ullmann coupling reaction is used only in symmetrical coupling reactions since mixtures are obtained from cross coupling reactions.[4]

Unsymmetric Ullmann couplings have been accomplished indirectly by connecting the two precursors into one molecule.[5] Chiral binaphthyl tethers proved successful in the synthesis of optically active biaryls. For symmetrical biaryls, the Ullmann reaction has an advantage over the oxazoline method, which requires the synthesis of two different precursors(eq. 1):[6]



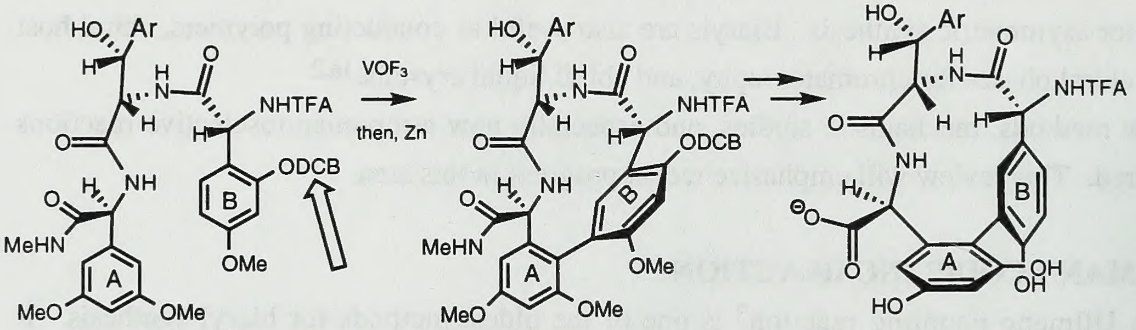
(1)

In this case the diastereoselectivity originates from thermodynamic control, but in the synthesis of binaphthyls kinetic control seems operative.[6b]

Copyright © 1994 by Xiping Su

OXIDATIVE COUPLING

Oxidative coupling mimics the biosynthesis of biaryl compounds. Although it is not uncommon for this reaction to produce mixtures of regioisomers, oxidative coupling often gives good yields in intramolecular coupling and sometimes is preferred for its simplicity.[7] Vanadium(V) and thallium(III) oxidants are more selective and effective with aromatic ethers. Evans employed VOF_3-induced biaryl coupling as the key step in the synthesis of the biphenyl macrocyclic tripeptide **5** in vancomycin(eq. 2).[8]



3 **4** **5**

(2)

Ar =3,5-dichloro-4-benzoyloxyphenyl,
DCB = 3,4-dichlorobenzyl.

The temporarily installed ortho substituent in B ring was crucial for the successful intramolecular coupling, but the $A^{1,3}$ strain caused by this added substituent was responsible for both the kinetic and thermodynamic bias for the undesired atropisomer. After removal of this group, thermodynamic equilibration favors the natural atropisomer.

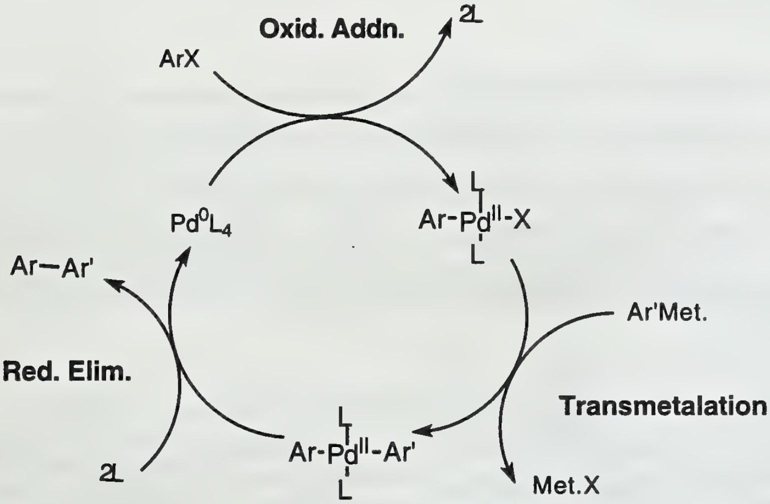
TRANSITION METAL-CATALYZED CROSS COUPLING REACTIONS

Suzuki Coupling

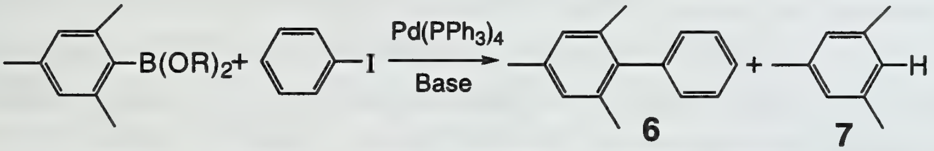
In 1981, Suzuki[9] first disclosed the coupling reaction between aryl boronic acids and aryl halides catalyzed by Pd^0. The mechanism,[10] which is similar to all transition metal-catalyzed cross-coupling reactions between organometallic reagents and electrophiles, involves three main steps: oxidative addition to Pd^0, transmetallation, and reductive elimination (Figure 1). Both monoaryl and biaryl palladium species were observed recently by electrospray ionization mass spectrometry.[10a]

Snieckus among others developed this methodology and it is widely used for the synthesis of sterically uncongested biaryls.[11] The Suzuki coupling reaction has a wide range of functional group compatibility; thus, nitro, carbonyl, hydroxyl and carboxyl groups are all tolerated. Besides aryl halides, aryl triflates proved to be a good partner in the coupling reaction as well.[12] This undoubtedly increases the practical importance for the Suzuki reaction because triflates are readily available from the corresponding phenols. The relative reactivity turned out to be: I > Br ≥ OTf >> Cl.

Figure 1


Oxid. Addn.
ArX
2L
Pd^0L_4
$Ar\text{-}Pd^{II}\text{-}X$ (L above and below Pd)
Ar—Ar'
Red. Elim.
Ar'Met.
Transmetalation
2L
$Ar\text{-}Pd^{II}\text{-}Ar'$ (L above and below Pd)
Met.X

Deboronation of aryl boronic acids may become a serious side reaction when electron-rich boronic acids are used[13]. Cross coupling efficiency can be greatly improved by optimization of reaction conditions(eq. 3).[14] 1,2-Dimethoxyethane as solvent decreases competing deboronation, and stronger bases, e.g., $Ba(OH)_2$, accelerate the reaction . Aprotic conditions are desirable for boronic acids or haloarenes sensitive to aqueous base.

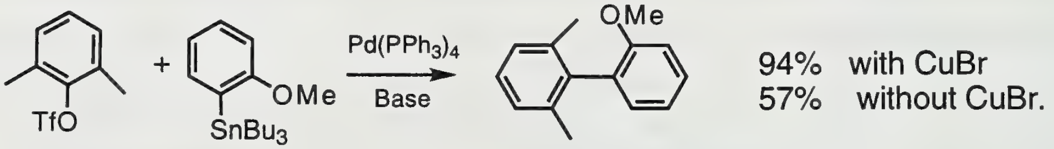


(3)

$(OR)_2$	Base	Solv.	Temp.	Time(hrs.)	Product Ratio
$(OH)_2$	Na_2CO_3	C_6H_6/H_2O	80	48	77 : 23
$(OH)_2$	$Ba(OH)_2$	DME/H_2O	80	4	98 : 2
$O(CH)_2O$	K_3PO_4	DMF	100	8	98 : 2

Stille Coupling of Arylstannanes

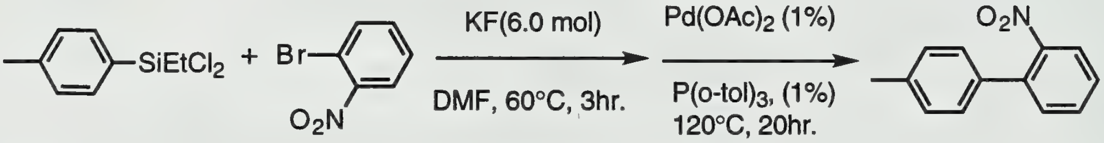
The Stille reaction is another widely used cross coupling method.[15] The conventional synthesis usually requires elevated temperature for complete reaction. Recently an elegant study[16] showed that changing the ligands on the palladium catalyst have a dramatic influence on the reaction and with proper choice of ligands, it was possible to achieve complete reaction at room temperature. Although this study used a vinylstannane, it may broaden the scope of the Stille aryl coupling as well. Copper(I) salts greatly increase the yield of biphenyls(eq. 4).[17]



(4)

Other Transition Metal-Catalyzed Couplings

Aryl halides and triflates react with arenes in the presence of Pd^0 to yield biaryls. Although regioisomers are generated, intramolecular coupling gives good yields of biaryl compounds.[18] Arylzinc and Grignard reagents[19] couple with aryl halides or triflates in the presence of Pd^0 or Ni^0. A promising cross coupling of aryl silanes with aryl bromides or iodides has also appeared(eq. 5).[20]

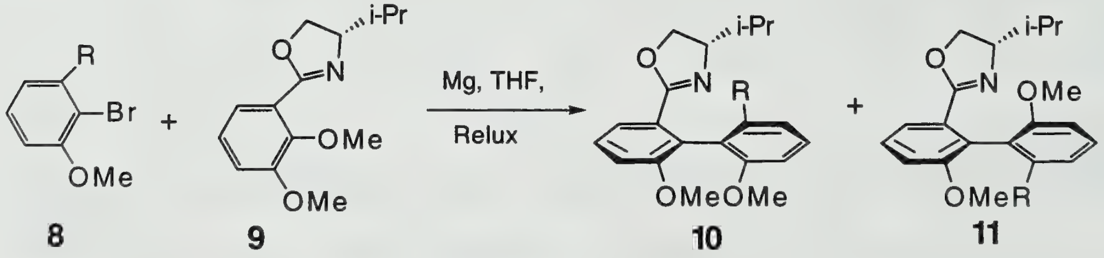


(5)

NUCLEOPHILIC DISPLACEMENT OF HETERO-SUBSTITUTED ARENES

Meyers' Oxazoline Method

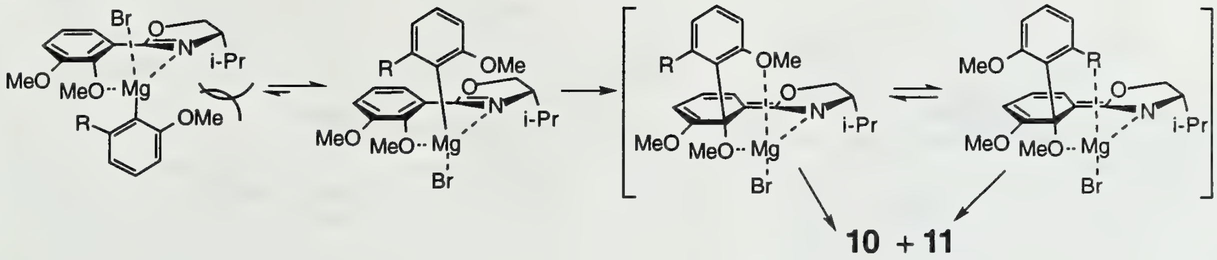
Meyers' oxazoline-mediated coupling is a well-developed method for biaryl synthesis.[21] The ready availability of chiral oxazolines and the steric insensitivity of the reaction are important advantages for the asymmetric synthesis of biaryl compounds. An example[22] is shown in eq. 6. It is apparent that non-chelating substituents gave the S isomer preferentially while chelating substituents resulted in poor diastereoselectivity. A chelation model explains these results satisfactorily(Figure 2).



(6)

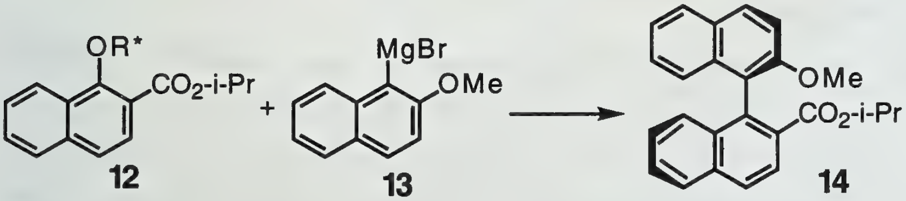
R	10		11
R = CH_3	90	:	10
CH_2OTBS	93	:	7
CH_2OCH_3	40	:	60

Figure 2



Other Nucleophilic Displacement of Grignard Reagents

Although Fuson[23] described the substitution of aromatic ethers activated by an ortho carboxyl group as early as 1956, this finding seems to have been ignored until recently. Miyano and coworkers found that binaphthyl compounds can be obtained in high enantiomeric excess when optically active menthyl esters(e.g. **14**) are displaced by Grignard reagents(eq. 7).[24]



R* = L-Menthyl

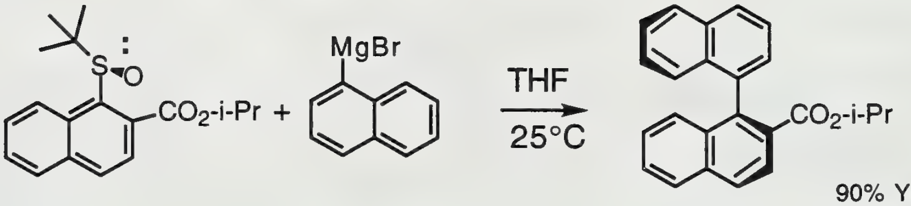
92% Yield,
97% e.e.

(7)

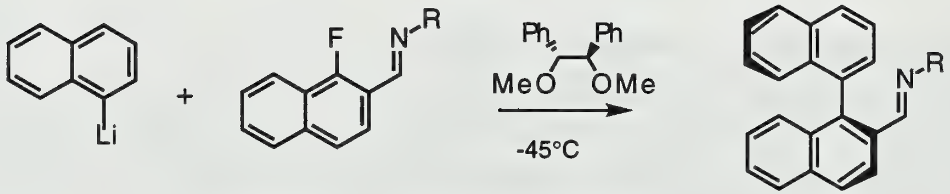
The mechanism resembles Meyers' coupling reaction. The chelation of magnesium to both the carbonyl and the methoxy group is very important for the reaction to proceed. For the synthesis of biphenyl carboxylates, isopropyl esters proved unsuitable because carbonyl addition predominated. The biphenyl products, however, can be obtained by using bulky mesityl, 2,6-diisopropylphenyl or even 2,6-di-t-butyl-4-methylphenyl esters.

Chiral sulfinyl groups have also been used as good leaving groups in the Grignard coupling reaction. The ortho carboxy group, which is required for the reaction, can be easily introduced by metallation. Excellent enantioselectivity can be achieved when optically active sulfoxides are employed(eq. 8).[25] Sterically more congested Grignard reagents failed to react. The mechanism is believed to proceed through a hypervalent sulfur intermediate.

A notable catalytic asymmetric synthesis was disclosed by Tomioka and coworkers.[26] The C_2 symmetric ligand, (S,S)-1,2-diphenyl-1,2-dimethoxyethane, promoted the coupling reaction between naphthyllithium and imine **17** (eq. 9). *N*-(2,6-Diisopropylphenyl)-imines were most selective. It was found that 1-naphthyllithium prepared free of lithium bromide was essential for high stereoselectivity. Although the scope of the reaction and the origin of the stereoselectivity remain to be explored, this promising result sheds some light on catalytic asymmetrical synthesis in this area.



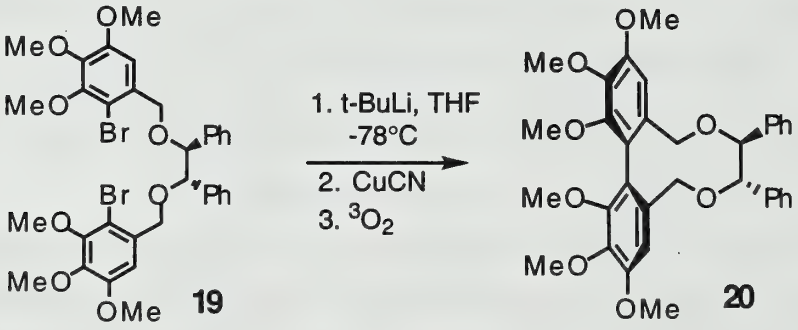
15 **16** 90% Yield 95% e.e. (8)



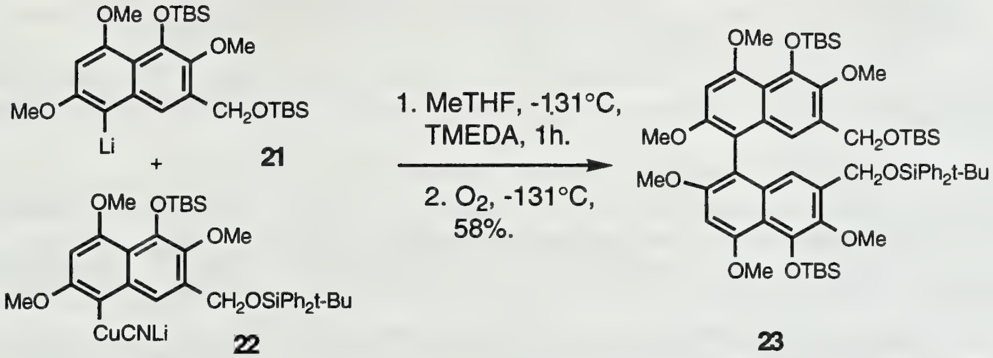
17 (R = 2,6 - i-Pr_2Ph-) 97% Yield 82% e.e. **18**

COUPLING OF CYANOCUPRATES

In their study to extend the synthetic utility of higher-order cyanocuprates,[27] Lipshutz and coworkers found that "kinetic" higher order cuprates gave high yields of cross-coupling products, in sharp contrast to the classical organocuprate coupling, which usually gives statistical mixtures of coupling products. The coupling reaction is insensitive to steric hindrance; for example, 2,2'-binaphthyls can be obtained in high yield. An application is the synthesis of (+)-*O*-permethyltellimagrandin II, in which diastereoselective intramolecular cyanocuprate coupling was the key step(eq. 10).[28] Since the reaction is conducted at very low temperature, sensitive substrates may tolerate the reaction conditions(eq. 11).[29] Suzuki and other couplings failed in this case.



19 **20** 77%, One isomer by NMR. (10)



(11)

MISCELLANEOUS METHODS

Bringmann and coworkers have developed an atrop-enantioselective ring opening of biaryl lactones.[30] Radical cyclization and electrosynthesis have also been used to synthesize biaryl compounds.[31]

CONCLUSION

Much progress has been made in biaryl synthesis in recent years, especially with atrop-enantioselective methods. Some questions remain to be answered, especially the mechanistic aspects and the origin of stereoselectivity. One can expect that more rational design of the reaction conditions, catalyst ligands, and even chiral catalysts for biaryl synthesis may be possible when the mechanisms are well-understood.

REFERENCES

1. For reviews, see: (a)Bringmann G.; Walter, R.; Weirich, R. *Angew. Chem., Int. Ed. Engl.* **1990,** *29*, 977. (b)Knight, D.W. In *Comprehensive Organic Synthesis*; Trost, B.M.; Fleming, I. eds.; Pergamon Press: Oxford, 1991, 481. For a computational study on the rotation of binaphthyl bonds, see: (c)Kranz, M.; Clark, T.; Schleyer, P.R. *J. Org. Chem.* **1993,** *58*, 3317.

2. (a)Noyori, R.; Takaya, H. *Acc. Chem. Res.* **1990,** *23*, 345. (b)Whitesell, J.K. *Chem. Rev.* **1989,** 1581. For biaryl natural products, see: (c)Hashimoto, T.; Yoshida, T.; Kan, Y.; Takaoka, S.; Tori, M.; Asakawa, Y. *Tetrahedron Lett.* **1994,** *35*, 909. For conducting polymers, see: (d)Roncali, J. *Chem. Rev.* **1992,** *92*, 711.

3. Sainsbury, M. *Tetrahedron*, **1980,** *36*, 3327.

4. See, however: Shimizu, N.; Kitamura, T.; Watanabe, K.; Yamaguchi, T.; Shigyo, H.; Ohta, T. *Tetrahedron Lett.* **1993,** *34*, 3421.

5. (a)Takahashi, M.; Kuroda, T., Ogiku, T.; Ohmizu, H.; Kondo, K.; Iwasaki, T. *Tetrahedron Lett.*, **1991,** *32*, 6919. (b)Takahashi, M.; Kuroda, T., Ogiku, T.; Ohmizu, H.; Kondo, K.; Iwasaki, T. *Heterocycles*, **1993,** *36*, 1867.(c)Miyano, S.; Fukushima, H.; Handa, S.; Ito, H.; Hashimoto, H. *Bull. Chem. Soc. Jpn.* **1988,** *61*, 3249.

6. (a)Nelson, T.D.; Meyers, A.I. *Tetrahedron Lett.* **1993,** *34*, 3061. (b)Nelson, T.D.; Meyers, A.I. *J. Org. Chem.* **1994,** *59* , 2655. (c)Nelson, T.D.; Meyers, A.I. *Tetrahedron Lett.* **1994,** *35*, 3259. (d)Meyers, A.I.; Meier, A.; Rawson, D.J. *Tetrahedron Lett.* **1992,** *33*, 853.

7. (a)Feldman, K.S.; Ensel, S.M.; Minard, R.D. *J. Am. Chem. Soc.* **1994,** *116*, 1742. (b)Mohr, B.; Enkelmann, V.; Wegner, G. *J. Org. Chem.* **1994,** *59*, 635. (c)Galan, A; Sutherland,

A.J.; Ballester, P.; Rebek, Jr., J. *Tetrahedron Lett.* **1994,** *35*, 5359. (d)Tanaka, M.; Mitsuhashi, H.; Wakamatsu, T. *Tetrahedron Lett.* **1992,** *33*, 4161.

8. (a)Evans, D.A.; Dinsmore, C.J.; Evrard, D.A.; DeVries, K.M. *J. Am. Chem. Soc.* **1993,** *115*, 6426. For a related study, see: (b)Rao, A.V.R.; Reddy, K.L.; Reddy, M.M. *Tetrahedron Lett.* **1994,** *35*, 5039.

9. Miyaura, N.; Yanagi, T.; Suzuki, A. *Synth. Commun.* **1981,** *11,* 513.

10. (a)Aliprantis, A.O.; Canary, J.W. *J. Am. Chem. Soc.* **1994,** *116*, 6985. (b)O'Keefe, D.F.; Dannock, M.C.; Marcuccio, S.M. *Tetrahedron Lett.* **1992,** *33*, 6679.

11. (a)Snieckus, V. *Chem. Rev.* **1990,** *90*, 879. (b)Martin, A.R.; Yang, Y. *Acta Chem. Scand.* **1993,** *47,* 221.

12. Oh-e, T.; Miyaura, N.; Suzuki, A. *J. Org. Chem.* **1993,** *58*, 2201.

13. Brandao, M.A.F.; de Oliveira, A.B.; Snieckus, V. *Tetrahedron Lett.* **1993,** *34*, 2437.

14. Watanabe, T.; Miyaura, N.; Suzuki, A. *Synlett* **1992,** 207.

15. (a)Stille, J.K. *Angew. Chem. Int. Ed. Engl.* **1986,** *25*, 508. (b)Mitchell, T.N. *Synthesis* **1992,** 803.

16. Farina, V.; Krishnan, B. *J. Am. Chem. Soc.* **1991,** *113,* 9585.

17. (a)Saa, J.M.; Martorell, G. *J. Org. Chem..* **1993,** *58*, 1963. (b)Kelly, T.R.; Xu, W.; Ma, Z.; Li, Q.; Bhushan, V. *J. Am. Chem. Soc.* **1993,** *115*, 5843. (c)Kelly, T.R.; Kim, M.H. *J. Org. Chem.* **1992,** *57*, 1593. (d)Deshpande, M.S. *Tetrahedron Lett.* **1994,** *35*, 5613. (e)Patel, H.K.; Kilburn, J.D.; Langley, G.J.; Edwards, P.D.; Mitchell, T.; Southgate, R. *Tetrahedron Lett.* **1994,** *35*, 481.

18. Hosoya, T.; Takashiro, E.; Matsumoto, T.; Suzuki, K. *Tetrahedron Lett.* **1994,** *35,* 4591.

19. (a)Amat, M.; Hadida, S.; Bosch, J. *Tetrahedron Lett.* **1994,** *35*, 793. (b)Uemura, M.; Nishimura, H.; Kamikawa, K.; Nakayama, K.; Hayashi, Y. *Tetrahedron Lett.* **1994,** *35*, 1909. (c)Takagi, K. *Chem. Lett.*; **1993,** 469.

20. (a)Hiyama, T.; Hatanaka, Y. *Pure Appl. Chem.* **1994,** *66*, 1471. (b)Hatanaka, Y.; Fukushima, S.; Hiyama, T. *Heterocycles*, **1990,** *30*, 303. (c) Hatanaka, Y.; Fukushima, S.; Hiyama, T. *Chem. Lett.*, **1989,** 1711.

21. Gant, T.G.; Meyers, A.I. *Tetrahedron,* **1994,** *50,* 2297.

22. (a)Moorlag, H.; Meyers, A.I. *Tetrahedron Lett.* **1993,** *34*, 6989. (b)Warshawsky, A.M.; Meyers, A.I. *J. Am. Chem. Soc.* **1990,** *112*, 8090.

23. Fuson, R.C.; Wassmundt, F.W.; *J. Am. Chem. Soc.* **1956,** *78*, 5409.

24. (a)Hattori, T.; Hotta, H.; Suzuki, T.; Miyano, S. *Bull. Chem. Soc. Jpn.* **1993,** *66*, 613. (b)Hattori, T.; Suzuki, T.; Hayashizaka, N.; Koike, N.; Miyano, S. *Bull. Chem. Soc. Jpn.* **1993,** *66*, 3034.

25. (a)Baker, R.W.; Pocock, G.R.; Sargent, M.V.; Twiss, E. *Tetrahedron: Asymmetry* **1993,** *4,* 2423. (b)Baker, R.W.; Pocock, G.R.; Sargent, M.V. *J. Chem. Soc., Chem. Commun.* **1993,** 1489.

26. Shindo, M.; Koga, K.; Tomioka, K. *J. Am. Chem. Soc.* **1992,** *114,* 8732.

27. For a review of organocuprates, see: Lipshutz, B.H.; Sengupta, S. *Org. React.* **1992,** *41*, 135.

28. (a)Lipshutz, B.H.; Liu, Z.-P.; Kayser, F. *Tetrahedron Lett.* **1994,** *35*, 5567. (b)Lipshutz, B.H.; Siegmann, K.; Garcia, E.; Kayser, F. *J. Am. Chem. Soc.* **1993,** *115*, 9276. (c)Lipshutz, B.H.; Siegmann, K.; Garcia, E. *Tetrahedron* **1992,** *48*, 2579. (d)Lipshutz, B.H.; Siegmann, K.; Garcia, E.; Kayser, F. *J. Am. Chem. Soc.* **1991,** *113*, 8161. For a discussion of higher order cuprates, see: (e)Bertz, S.H. *J. Am. Chem. Soc.* **1990,** *112,* 4031. (f)Lipshutz, B.H.; Sharma, S.; Ellsworth, E.L. *J. Am. Chem. Soc.* **1990,** *112,* 4032

29. Coleman, R.S.; Grant, E.B. *Tetrahedron Lett.* **1993,** *34,* 2225.

30. (a)Bringmann, G.; Hartung, T. *Liebigs Ann. Chem.* **1994,** 313. (b)Bringmann, G.; Hartung, T. *Angew. Chem. Int. Ed. Engl.* **1992,** *31*, 761. (c)Bringmann, G.; Hartung, T.; Krocher, O.; Gulden, K.-P.; Lange, J.; Burzlaff, H. *Tetrahedron Lett.* **1994,** *50*, 2831.

31. (a)Estevez, J.C.; Villaverde, M.C.; Estevez, R.J.; Castedo, L. *Tetrahedron* **1994,** *50,* 2107. (b)Boy, P.; Combellas, C.; Thiebault, A. *Tetrahedron Lett.* **1992,** *33*, 491.

SITE-SELECTIVE HYDROLYTIC CLEAVAGE OF PEPTIDES WITH TRANSITION METAL COMPLEXES

Reported by Eileen Thiessen September 12, 1994

INTRODUCTION

Proteins and peptides can be selectively cleaved by a variety of reagents, including proteolytic enzymes, cyanogen bromide, *o*-iodosobenzoic acid, and hydroxylamine. However, there is a continuing need for the development of reagents with selectivities different from those currently in use. In addition, reagents effective under physiological conditions are required by biochemists trying to elucidate the tertiary or quartenary structure of proteins. Hydrolytic reagents are generally preferred over oxidative ones because hydrolyzed peptides can be more easily re-ligated and oxidative reagents can cause side reactions.

Coordination complexes of transition metals have potential as artificial metalloproteases. They tend to be simple to prepare, they can be small enough to cleave proteins without disrupting the tertiary structure, and the ligands can be varied to enhance selectivity or rates. Many proteolytic enzymes contain transition metals; the active sites of these enzymes can be used as a basis for the design of artificial complexes. This review will present different approaches to understanding the mechanism of amide hydrolysis and the factors that determine selectivity of peptide cleavage mediated by transition metal complexes.

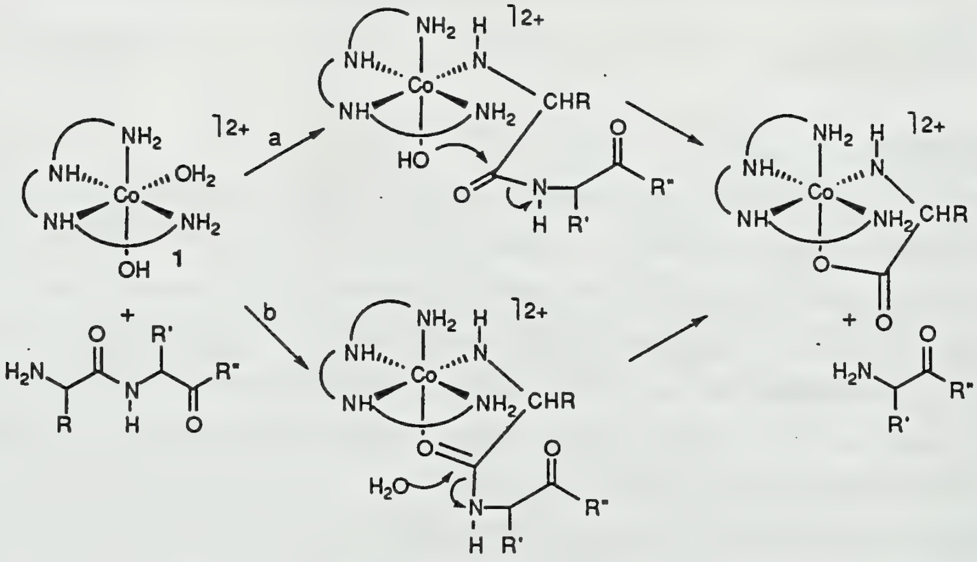
BACKGROUND

Kroll showed in 1952[1] that divalent metal ions accelerate the rate of hydrolysis of amino acid esters. Li and co-workers[2] studied the interactions between metals and amino acids or peptides. Further work by Bender and Turnquest[3] examined the kinetics and mechanism of cupric ion-catalyzed hydrolysis of α-amino acid esters. In 1956, Meriwether and Westheimer[4] showed that divalent transition metal cations accelerate the hydrolysis of peptides. However, in all of these reactions, hydrolysis apparently took place in an indiscriminate manner.

In 1963, Collman and Buckingham[5] reported the selective hydrolysis of the N-terminus of simple peptides by *cis*-$[Co(trien)OH(OH_2)]^{2+}$ (**1**, trien=triethylenetetramine). In the first of two proposed mechanisms (Scheme I), the amino group displaces a water molecule and coordinates to cobalt. An adjacent hydroxyl or aquo ligand then attacks the peptide carbonyl. In the second mechanism, the N-terminal amino group forms a N,O-chelate, activating the carbonyl for attack by external hydroxide. The β configuration of the trien ligand is shown to illustrate the two proposed mechanisms.

Copyright © 1994 by Eileen Thiessen

Scheme I. Possible mechanisms for peptide hydrolysis promoted by Co(III) complexes



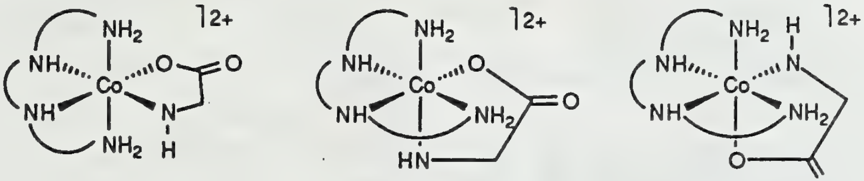
Cobalt(III), like divalent metals, can enhance the reactivity of coordinated ligands. However, the ligands are less labile and reactions tend to be stoichiometric rather than catalytic, allowing researchers to monitor the reactions and isolate intermediates, which facilitates the determination of the mechanism.[6] Such reactions have been done with Co(III) complexes.[7] Because of the complexities involved in investigating the hydrolysis of proteins, researchers have studied simpler substrates such as esters and amides, derivatives of amino acids and small peptides.

MECHANISTIC STUDIES ON HYDROLYSIS BY COBALT(III)

To examine how ligands affect hydrolysis, Buckingham and Collman studied $[Co(en)_2OH(H_2O)]^{2+}$ (en=ethylenediamine).[7e] Reactions promoted by this complex resulted in multiple products, including more than one metal complex. The slow rates necessitated rather high temperatures , and the diversity of products rendered reaction monitoring and characterization of intermediates difficult. These problems were attributed to several factors, the most significant being complications resulting from cis-trans isomerization of $[Co(en)_2OH(H_2O)]^{2+}$. They hypothesized that a tetradentate ligand would force the aquohydroxo groups into cis configuration.[8] Cis chelation of either or both the peptide and the hydrolyzed product was believed to be necessary for reaction to occur. *cis*-$[Co(trien)OH(H_2O)]^{2+}$ (**1**) was thus chosen for further study.[7d]

The reaction of **1** with peptides, amino acid esters, and amino acid amides in the pH range 7.5-8.0 yielded the corresponding amino acid complexes $[Co(trien)AA]^{2+}$ (AA=amino acid anion). This type of complex has three geometric isomers, one of which has the triethylenetetramine ligand

Figure I. Three possible geometrical structures of $[Co\text{-}(trien)gly]^{2+}$



α β₁ β₂

in a symmetrical D*-α configuration[†] and two of which have the ligand in an asymmetrical L*-β configuration (Figure I).[7d,9] Since the D*-α configuration is known to isomerize to the L*-β form under basic conditions, only the L*-β complex was used. A mixture of the two β complexes, formed upon reaction of $[Co(trien)OH(H_2O)]^{2+}$ with glycine, was used to study this mechanism.

In reactions of the β form of **1** (β-**1**) with different substrates, spectroscopic evidence showed that the configuration was retained.[7a] With equimolar amounts of β-**1** and peptide, the complex $[Co(trien)AA]^{2+}$ formed selectively with the N-terminal amino acid. When the N-terminus of the peptide was acylated (e.g. with carbobenzoxy), **1** failed to cleave any of the bonds under the usual conditions. Thus, coordination of the free amino group is required for hydrolysis to occur.

While these studies are not conclusive towards either of the aforementioned mechanisms, they reveal that the source of the selectivity is in the ability of a free N-terminal amino group to bind to the metal complex. However, this selectivity has not been tested on peptides containing residues with side chains that may chelate to the metal along with the N-terminus.

MODELS OF CARBOXYPEPTIDASE A

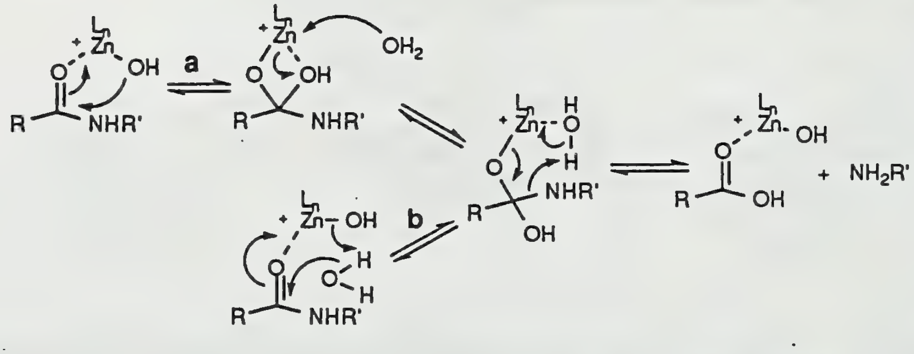
Carboxypeptidase A (CPA) is a zinc metalloenzyme which catalyzes the hydrolysis of the C-terminal amino acid of polypeptides. Researchers hope not only to determine the mechanism of action of this enzyme, but also to understand structural and reactivity features that can be used in designing selective artificial metalloenzymes.

Several mechanisms have been proposed for the formation of the tetrahedral intermediate (TI).[10-13] It is hoped that insight into the kinetics and mechanism of the enzyme might be gained through understanding TI formation. Sayre has raised the possibility that increasing only the efficiency of TI formation may not increase the rate of amide hydrolysis. Model studies[14] have shown TI formation in ester hydrolysis to be the rate-limiting step. But since amide hydrolysis

---

[†] D* refers to the positive (dextro) specific rotation, and a positive Cotton effect in the optical rotatory dispersion (O.R.D.) curve for the first Co(III) band. L* refers to negative (levo) specific rotation, and a positive Cotton effect.

Scheme II



involves a poor leaving group (formally RNH$^-$), it is the breakdown (C-N bond cleavage) of the TI that is rate-limiting.[15] To explain the rapid rate of amide hydrolysis by zinc peptidases, Sayre postulates that metal complexes promote both the formation *and* the breakdown of the TI.[16]

The rate of C-N cleavage in the TI can be increased by making the amine a better leaving group. At low pH, protons catalyze rapid expulsion of the amine. At physiological pH, an efficient general acid catalyst (GAC) would be required to catalyze the process. Both internal and external GACs have been shown to enhance greatly amide hydrolysis.[17,18] In some cases, this rate increase is so great that TI formation becomes rate-limiting.

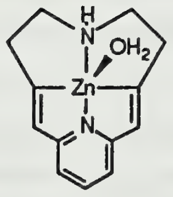
Thus, an efficient catalyst for amide hydrolysis would be able to promote both TI formation and TI breakdown. According to Sayre, Zn promotes TI formation in two ways: under physiological conditions, the zinc ion coordinates to water to form zinc hydroxide (Zn-OH). Zn-OH is a better nucleophile and serves to stabilize the TI (Scheme IIa). Alternatively, Zn-OH could serve as a general base to assist water as a nucleophile (Scheme IIb). Zn-OH_2 could also serve as a GAC to break down the TI.

These theories seem consistent with the studies of Groves[18] and Parkin,[19] although much work remains to apply the ideas in the design of artificial proteases that are as efficient and selective as CPA.

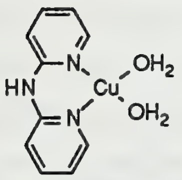
CIS DIAQUO COMPLEXES

In a search for artificial metalloenzymes, Chin and co-workers have studied the hydrolysis of peptides and amino acid derivatives, as well as the hydrolysis of esters, nitriles, phosphate monoesters, and diesters with zinc, copper, and cobalt complexes (**2**, **3**, and **4** respectively).[20] The ultimate objective is to design artificial metalloenzymes which can selectively cleave any of these substrates.

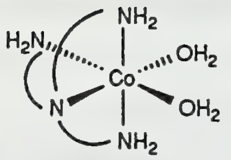
Initial studies focused on ester hydrolysis, which is mechanistically similar to amide hydrolysis. Simple catalysts such as imidazole are as effective as hydroxide ion at catalyzing the



2



3

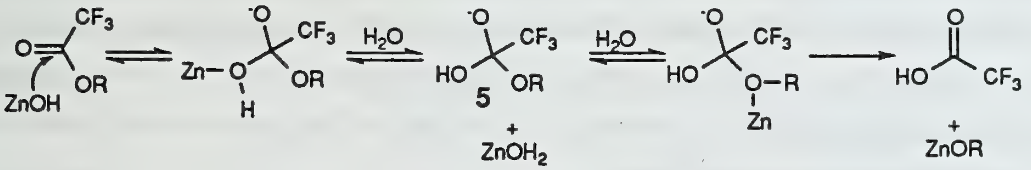


4a *cis*-$[(trpn)Co(OH_2)_2]^{3+}$
4b *cis*-$[(tren)Co(OH_2)_2]^{3+}$

hydrolysis of esters with good leaving groups (e.g., *p*-nitrophenoxide, acetate), but millions of times slower than hydroxide for catalyzing the hydrolysis of esters with poor leaving groups (e.g., methoxide).[21] Surprisingly, they found that zinc hydroxide complex **2** catalyzes the hydrolysis of methyl trifluoroacetate but is nearly unreactive towards methyl acetate, even though both leaving groups are unactivated methoxide.[22]

The catalytic mechanism should afford an understanding of the more rapid hydrolysis of methyl trifluoroacetate. The two most likely mechanisms are (1) the Lewis acid coordination, in which the zinc ion activates the carbonyl carbon for nucleophilic attack by external hydroxide, and (2) the metal hydroxide mechanism, in which a metal hydroxide attacks the ester. The former mechanism is ruled out on the basis of the reactivity-selectivity principle,[23,24] leaving the metal hydroxide mechanism (Scheme III).

Scheme III

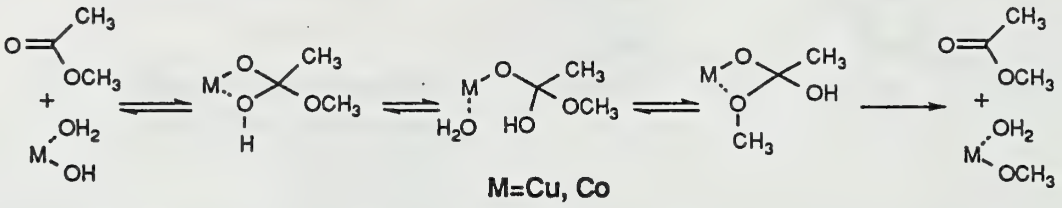


The lifetime of TI **5** is much longer for methyl trifluoroacetate than that for methyl acetate.[25] If **5** is too short-lived for zinc to exchange, then the reaction cannot go to completion. The use of a cis diaquo species as catalyst (Scheme IV) would extend the lifetime of the TI *via* four-membered ring formation, and the anionic oxygen of the TI would always be coordinated to the metal, making metal migration intramolecular and thus more efficient. Indeed, Chin and co-workers found that **3** catalyzed the hydrolysis of methyl acetate, as well as amides under mild conditions.[26]

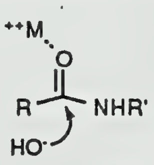
Cis diaquo Co(III) complexes also catalyze the hydrolysis of unactivated esters. *cis*-$[(trpn)Co(OH_2)_2]^{3+}$ (**4a**, trpn=tris(aminopropyl)amine) catalyzes the hydrolysis of amides and methyl acetate, while *cis*-$[(tren)Co(OH_2)_2]^{3+}$ (**4b**, tren=tris(aminoethyl)amine) is inactive towards both. This reactivity difference is attributed to the formation of the four-membered ring (see

Scheme IV): **2** and **4a** form four-membered chelates with acetate whereas **4b** is inactive because it forms only a monodentate complex.

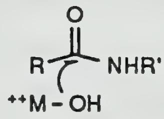
Scheme IV



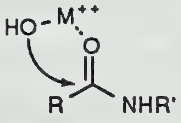
If the hydrolysis of amides follows the same pattern, then the metal hydroxide mechanism (**7**) would be favored over the Lewis acid mechanism (**6**). However, since Groves[18] and Breslow[27] showed the feasibility of either mechanism in model studies of CPA, Chin suggested combining both into a single mechanism (**8**).



6



7



8

The catalytic efficiencies of **2** and **3** for formamide hydrolysis were compared. Complex **2** cannot follow mechanism **8** because it is only a monoaquo species. If **8** is the favored mechanism, then **2** should be less efficient than **3** in promoting formamide hydrolysis. If either **6** or **7** is favored, then **2** and **3** should be equally efficient. Catalysis by the diaquo complex was found to be over two orders of magnitude more efficient, supporting mechanism **8**. Thus, it appears that similar to ester hydrolysis, the most efficient catalysts are cis diaquo complexes, which have the ability to form stable four-membered rings.

Chin has studied in great depth the factors that govern the rate of amide hydrolysis, and has achieved large rate enhancements with cis diaquo complexes. However, the question of site-selectivity in polypeptide hydrolysis remains to be addressed.

IRON CHELATE-MEDIATED PROTEOLYSIS

Recently, Rana and Meares[28] reported the specific cleavage of the proteins bovine serum albumin (BSA) and human carbonic anhydrase I (HCAI) by an attached iron chelate.[29] Control experiments showed that cleavage was very specific and that Fe^{2+}, 1-(*p*-bromoacetamidobenzyl)-EDTA, H_2O_2, and ascorbate were all required for the reaction. Cleavage did not occur if the chelate was not attached to the protein. The process is apparently catalytic since for BSA each molecule of chelate caused cleavage at two sites. Also, $[^{18}O]H_2O_2$ and $[^{18}O]O_2$ labeling experiments led Rana and Meares to propose a highly nucleophilic oxygen species, such as iron-coordinated peroxide, that attacks a nearby carbonyl. Specificity is governed by proximity and orientation of the chelate to a peptide bond rather than by amino acid residue type.

This method of utilizing a bifunctional chelate as a site-specific protease provides a good method for characterizing the structure of proteins under physiological conditions. Since the selectivity is determined by the proximity of the chelate to the cleavage sites, controlling the size and shape of the chelate, as well as the anchoring site could lead to controlled cleavages of a protein.

CONCLUSIONS

Although many reagents are available for protein and peptide cleavage, new and better reagents which can increase the rate and selectivity of proteolysis are always in demand. Collman and Buckingham demonstrated the selective hydrolysis of the *N*-terminus of simple peptides by Co(III) complexes; further work should address more complex peptides containing amino acid residues that can compete with binding of the *N*-terminus to the complex. Sayre investigated zinc complexes and theorized about factors which may affect the rate of amide hydrolysis. These ideas remain to be tested. Large rate enhancements for amide hydrolysis with cis diaquo species have been achieved by Chin, but the question of selectivity has not yet been addressed. Rana and Meares' iron chelate shows great promise as a way to investigate the tertiary structure of a protein. Future work could include developing chelates to bind selectively at various sites of a protein in order to control cleavage. Although artificial metalloproteases have not yet reached the efficiency of their natural analogs, the research described here furnishes understanding that will help to narrow the gap.

REFERENCES

1. (a) Kroll, H. *J. Am. Chem. Soc.* **1952**, *74*, 2034. (b) Kroll, H. *J. Am. Chem. Soc.* **1952**, *74*, 2036.
2. (a) Li, N. C.; Manning, R. A. *J. Am. Chem. Soc.* **1955**, *77*, 5225. (b) White, J. M.; Manning, R. A.; Li, N. C. *J. Am. Chem. Soc.* **1956**, *78*, 2367. (c) Li, N. C.; Doody, B. E.; White, J. M. *J. Am. Chem. Soc.* **1957**, *79*, 5859. (d) Mathur, R.; Li, N. C. *J. Am. Chem. Soc.* **1964**, *86*, 1289.
3. Bender, M. L.; Turnquest, B. W. *J. Am. Chem. Soc.* **1957**, *79*, 1889.
4. Meriwether, L.; Westheimer, F. H. *J. Am. Chem. Soc.* **1956**, *78*, 5119.
5. Collman, J. P.; Buckingham, D. A. *J. Am. Chem. Soc.* **1963**, *85*, 3039.
6. Sutton, P. A.; Buckingham, D. A. *Acc. Chem. Res.* **1987**, *20*, 357.

7. (a) Buckingham, D. A.; Jones, D. *Inorg. Chem.* **1965**, *4*, 1387. (b) Collman, J. P.; Schneider, P. W. *Inorg. Chem.* **1966**, *5*, 1380. (c) Collman, J. P.; Kimura, E. *J. Am. Chem. Soc.* **1967**, *89*, 6096. (d) Buckingham, D. A.; Collman, J. P.; Happer, D. A. R.; Marzilli, L. G. *J. Am. Chem. Soc.* **1967**, *89*, 1082. (e) Buckingham, D. A.; Collman, J. P. *Inorg. Chem.* **1967**, *6*, 1803.
8. Searle, G. H., Ph.D. Thesis, Australian National University, Canberra, A. C. T., **1964**.
9. (a) Kyuno, E.; Boucher, L. J.; Bailar, J. C., Jr. *J. Am. Chem. Soc.* **1965**, *87*, 4458. (b) Kyuno, E.; Bailar, J. C., Jr. *J. Am. Chem. Soc.* **1966**, *88*, 1121. (c) Kyuno, E.; Bailar, J. C., Jr. *J. Am. Chem. Soc.* **1966**, *88*, 1125.
10. (a) Makinen, M. W.; Kuo, L. C.; Dymowski, J. J.; Jaffer, S. *J. Biol. Chem.* **1979**, *254*, 356. (b) Kuo, L. C.; Makinen, M. W. *J. Biol. Chem.* **1982**, *257*, 24. (c) Suh, J.; Cho, W.; Chung, S. *J. Am. Chem. Soc.* **1985**, *107*, 4530. (d) Sander, M. E.; Witzel, H. *Biochem. Biophys. Res. Commun.* **1985**, *132*, 681.
11. (a) Hammond, G. S. *J. Am. Chem. Soc.* **1955**, *77*, 334. (b) Auld, D. S.; Galdes, A.; Geoghegan, K. F.; Holmquist, B.; Martinelli, R. A.; Vallee, B. L. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 5041.
12. Christianson, D. W.; Lipscomb, W. N. *Acc. Chem. Res.* **1989** and references therein.
13. Fife, T. H.; Przystas, T. J. *J. Am. Chem. Soc.* **1986**, *108*, 4631.
14. (a) Martell, A. E. *Metal Ions Biol. Sys.* **1973**, *2*, 207. (b) Hay, R. W.; Morris, P. J. *Metal Ions Biol. Sys.* **1974**, *5*, 173. (c) Hay, R. W.; Clark, C. R. *J. Chem. Soc., Dalton Trans.* **1977**, 1866. (d) Fife, T. H.; Prystas, T. J. *J. Am. Chem. Soc.* **1985**, *107*, 1041.
15. (a) O'Connor, C. *Q. Rev., Chem. Soc.* **1970**, *24*, 553. (b) Pollack, R. M.; Bender, M. L. *J. Am. Chem. Soc.* **1970**, *92*, 7190. (c) Kershner, L. D.; Schowen, R. L. *J. Am. Chem. Soc.* **1971**, *93*, 2014.
16. Sayre, L. M. *J. Am. Chem. Soc.* **1986**, *108*, 1632.
17. For more details, see references 9 and 10 of reference 16.
18. Groves, J. T.; Baron, L. A. *J. Am. Chem. Soc.* **1989**, *111*, 5442.
19. Looney, A.; Han, R.; McNeill, K.; Parkin, G. *J. Am. Chem. Soc.* **1993**, *115*, 4690.
20. Chin, J. *Acc. Chem. Res.* **1991**, *24*, 145 and references therein.
21. Jencks, W. P.; Kirsch, J. F. *J. Am. Chem. Soc.* **1964**, *86*, 827.
22. Chin, J.; Zou, X. *J. Am. Chem. Soc.* **1984**, *106*, 3687.
23. Lowry, T. H.; Richardson, K. S. *Mechanism and Theory in Organic Chemistry*, 3rd ed.; Harper & Row: New York, 1987; p. 148.
24. See explanation on p. 146 of reference 20.
25. Guthrie, J. P.; Cullimore, P. A. *Can. J. Chem.*1980, *58*, 1281.
26. Chin, J.; Jubian, V.; Mrejen, K. *J. Chem. Soc., Chem. Commun.* 1990, 1326.
27. Schepartz, A.; Breslow, R. *J. Am. Chem. Soc.* **1987**, *109*, 1814.
28. Rana, T. M.; Meares, C. F. *J. Am. Chem. Soc.* **1990**, *112*, 2457.
29. (a) Rana, T. M.; Meares, C. F. *J. Am. Chem. Soc.* **1991**, *113*, 1859. (b) Rana, T. M.; Meares, C. F. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 10578.

CHIRAL ACYL NITROSO COMPOUNDS IN ASYMMETRIC SYNTHESIS

Reported by David Gordon September 29, 1994

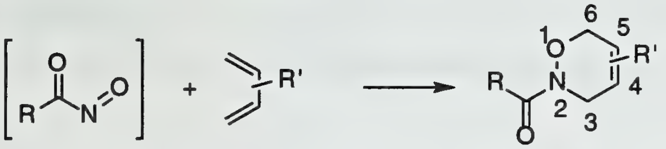
INTRODUCTION

The reactions and synthetic applications of acyl nitroso compounds have been investigated extensively since their intermediacy was first proposed.[1] These nitroso compounds are highly electrophilic dienophiles, which react with dienes in [4+2] cycloadditions, and the regiochemistry and stereoselectivity has been well investigated through several synthetic studies.[1a,b] Recently, the asymmetry induced by chiral acyl nitroso compounds has been the subject of much interest.[1e] This paper first will review the regioselective and stereoselective factors involved with acyl nitroso reactions and then will discuss the asymmetric induction of chiral acyl nitroso compounds and applications in synthesis. Other related compounds, such as alkyl, aryl, α-chloro, cyano, vinyl, and imino nitroso compounds,[1] will not be covered.

BACKGROUND

Acyl nitroso compounds have been used in the synthesis of natural products, such as amino acids,[2] amino sugars,[3] and alkaloids.[4] The key to these syntheses is formation of an N-acyl-3,6-dihydro-1,2-dihydrooxazine, incorporating oxygen and nitrogen in a *cis*-1,4-bis-allylic arrangement (Scheme I). Most of these syntheses necessarily give racemic products because the starting materials are achiral.[3-5]

Scheme I

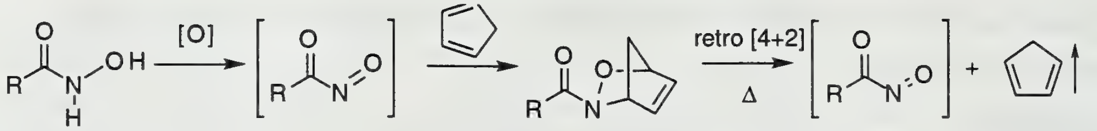


In related reactions using α-chloronitroso compounds,[1e] a chiral auxiliary on the dienophile was employed to induce asymmetry in the parent dihydrooxazine. Similarly, a chiral auxiliary as part of an acyl nitroso compound can affect the diastereomer ratio through steric effects.[6]

Generation of Acyl Nitroso Compounds

Acyl nitroso compounds are short-lived species that exist in solution in equilibrium with their azo dioxide dimer.[7] Unlike stable aryl and α-chloro nitroso compounds,[8] acyl nitroso compounds must be generated *in situ* from the corresponding hydroxamic acid, typically by oxidation with periodate or with the Swern or Corey-Kim reagents.[2,9] Acyl nitroso compounds can be trapped[10] and stored as either cyclopentadiene (eq 1) or a 9,10-dimethylanthracene (DMA) adducts. Thermal cracking (60 °C for cyclopentadiene, refluxing benzene for DMA) liberates the nitroso compounds by retro-Diels-Alder mechanism.

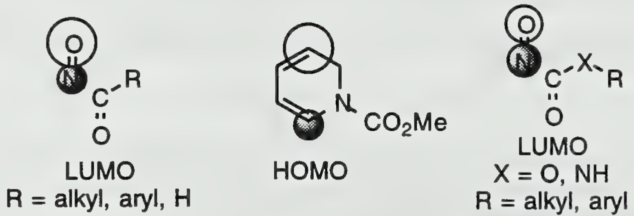
Copyright © 1994 by David A. Gordon



(1)

Regiochemistry

Acyl nitroso compounds are strong electrophiles because of the electron withdrawing effect of the carbonyl group on the nitroso functionality.[11] As a result, the LUMO of the acyl (i.e. alkyl/aryl carbonyl) nitroso species **1** has a large coefficient at oxygen and a small coefficient at nitrogen (Figure 1).[12] If the HOMO of the diene also is unsymmetrical (e.g. **2**), a prediction of the regiochemistry can be made. However, the LUMO's of alkoxycarbonyl and carbamoyl nitroso species **3** have similar coefficients at nitrogen and oxygen; consequently, little or no regioselectivity is expected in these cycloaddition reactions.

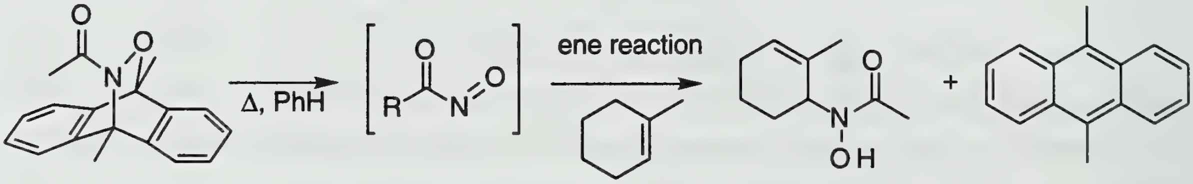


1 **2** **3**

Figure 1. Orbital coefficient comparisons

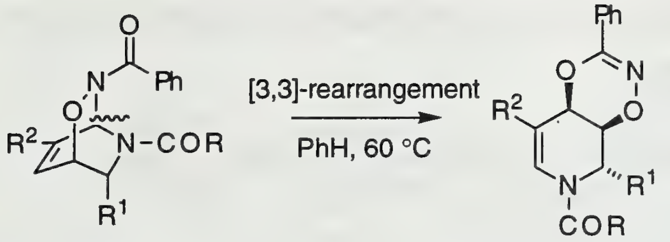
Related Acyl Nitroso Reactions

Occasionally acyl nitroso compounds do not act as dienes in [4+2] cycloadditions. At elevated temperatures (77-80 °C)[10] or in the absence of an available diene,[13] acyl nitroso compounds undergo the Alder ene reaction with olefins to give allylic hydroxamic acids (e.g. eq 2).



(2)

Alternatively, some dihydrooxazine products from [4+2] cycloadditions are thermally unstable and undergo [3,3]-sigmatropic rearrangements to yield 5,6-dihydro-1,2,4-dioxazines (eq 3).[14] The rearrangement most likely occurs by a step-wise mechanism. The nitroso functionality is probably not liberated during the reaction, since the addition of triphenylphosphine, a nitroso trapping reagent, did not inhibit the rearrangement.



(3)

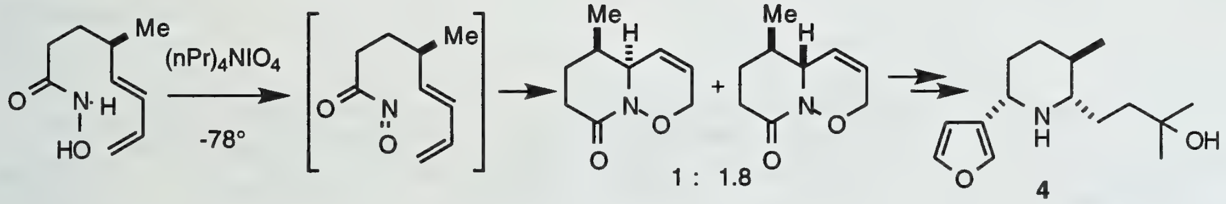
ASYMMETRIC INDUCTION

Acyl nitroso compounds bearing stereogenic centers will generate diastereomeric cycloadducts.[15] The asymmetric induction associated with intramolecular and intermolecular cycloadditions will be discussed in the following sections.

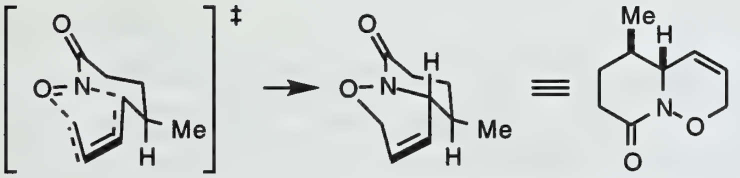
Intramolecular

In intramolecular Diels-Alder reactions, one stereogenic center can affect the facial selectivity.[6,16] (-)-Nupharamine (**4**) was synthesized (Scheme II) in this way from enantiomer-rich starting material, (R)-citronellol.[16] The kinetically favored cis stereochemistry was rationalized by a transition state in which the stereogenic substituent lies in a pseudo equatorial position (Scheme III).

Scheme II



Scheme III



Rotational conformations: syn versus anti

The rotational conformation of the acyl-nitroso C-N bond has been a subject of controversy. From MM+ force field calculations, the two lowest energy conformations are syn-planar or anti-planar (Figure 2). According to the Curtin-Hammett principle,[15] the conformation leading to the lowest energy transition state will lead to the major products, regardless of the ground state conformation. Kirby[17] and Proctor[18] calculated that the lowest energy state of the acyl nitroso compound has the anti conformation. Subsequently, Streith[19] and Ghosez[20] postulated that the major products arise from a transition state with a syn rotational conformation, based on *ab initio* LCAO-SCF-MO calculations using Huzinaga's MINI1 basis set.[20] These two differing rotational conformations have been used to explain the transition states that lead to the observed products.

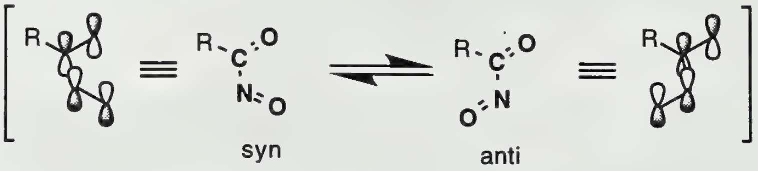
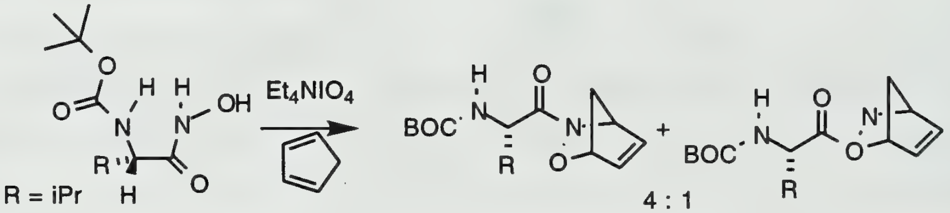


Figure 2. Syn versus anti rotational conformation

Internal Hydrogen Bonding

The rotational conformation of an acyl nitroso compound may be fixed in a particular transition state by internal noncovalent interactions. α-Amino acids[21] are examples of chiral auxiliaries that rely on internal hydrogen bonding to induce facial selectivity. The *N*-(BOC)-protected hydroxamic acids derived from amino acids react with cyclic dienes (eq 4) to form isomeric adducts with diastereoisomeric excesses ranging from 20% (L-phenylalanine) to 60% (L-valine) and with 60-90% yields of diastereomers. Either a syn or anti conformation (Figure 3) in the transition state can be used to explain the facial selectivity and the major diastereomer observed. The diene approaches from the less hindered face in an *endo* configuration in either case.



(4)

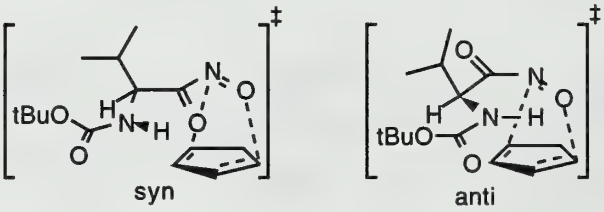
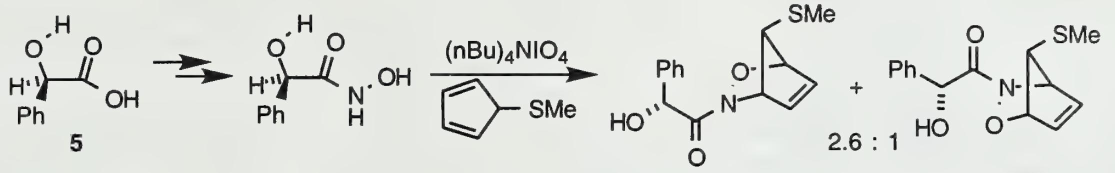
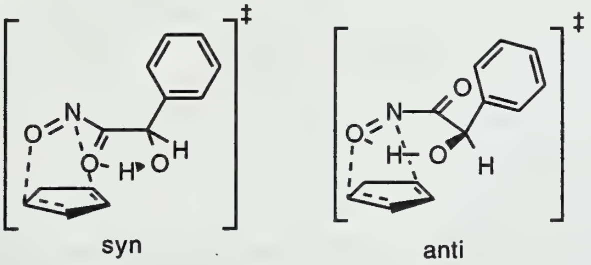


Figure 3. Amino acid auxiliaries: transition states - syn and anti

Internal hydrogen bonding has also been invoked to explain the diastereoselectivity when α-hydroxy acids are employed as chiral auxiliaries. One example is D-mandelic acid **5**, which was applied in the synthesis of mannosatin A **6**, a naturally-occuring α-mannosidase inhibitor.[24] Under oxidative conditions, D-mandelohydroxamic acid reacts with 5-methylthio-1,3-cyclopentadiene (eq 5) to yield a 2.6:1 mixture of diastereomers. Like the preceding acyl nitroso compounds from α-amino acids, the major diastereomer could arise from either an endo-syn or an endo-anti[9a] transition state, as discussed above for the α-N-acylamino analogs (Figure 4). In these models, the diene approaches from the less hindered face of the hydrogen bonded nitroso compound.



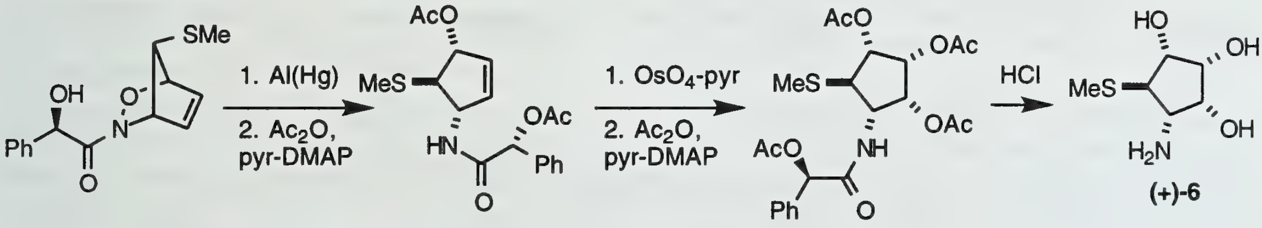
(5)



syn anti

Figure 4. Mandelic acid auxiliary: transition states - syn and anti

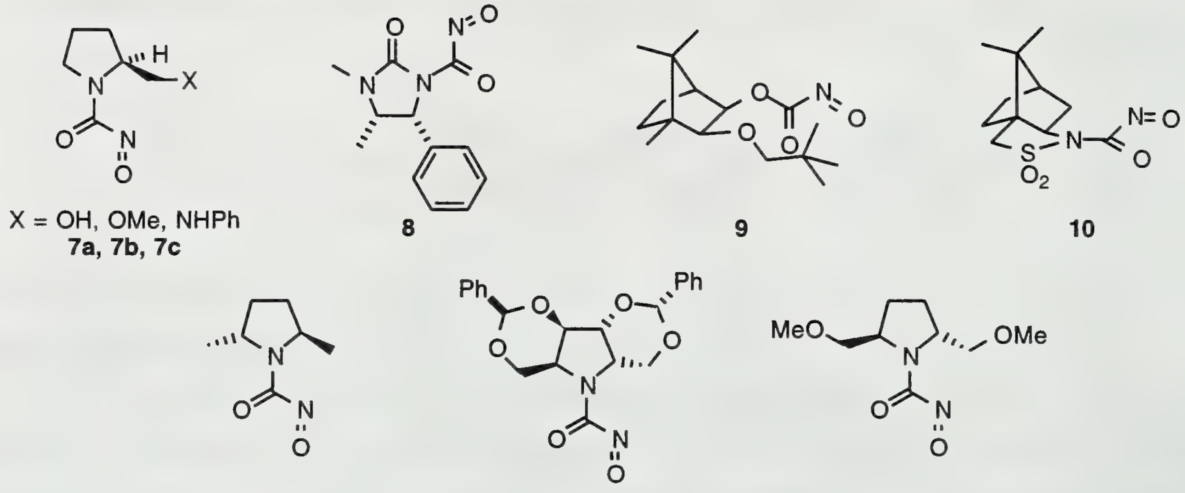
After separation of the major diastereomer, the N,O-bond of the bicyclic isoxazolidine is cleaved using aluminum amalgam, and then the 1,4-amino alcohol is diacetylated with acetic anhydride (eq 6).[24] Osmylation generated a vicinal diol, *cis* to the formerly bis-allylic substituents. Acetylation followed by acidic hydrolysis produced mannostatin A (**6**) as a hydrochloride salt.



(6)

Intermolecular: Steric Effects

Other chiral auxiliaries rely on steric factors to influence facial selectivity (Figure 5), instead of internal hydrogen bonding. Proline-based derivatives **7**,[25] imidazolidines **8**,[26] Oppolzer's camphor-based alcohol **9**,[27] bornane-sultam **10**,[20,28] and C$_2$-symmetric pyrrolidines **11**, **12**, **13**,[28,29] all induce moderate-to-high diastereoisomeric excesses (Table I). In each case except the pyrrolidines, the observed stereochemistry is consistent with the assumption of a syn carbonyl-nitroso conformation in the transition state.



X = OH, OMe, NHPh
7a, 7b, 7c

8

9

10

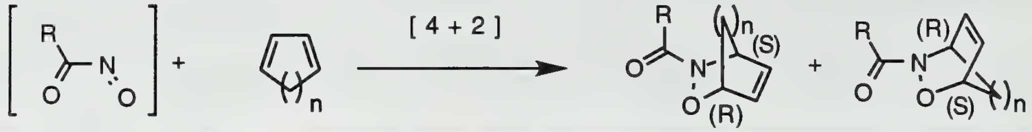
11

12

13

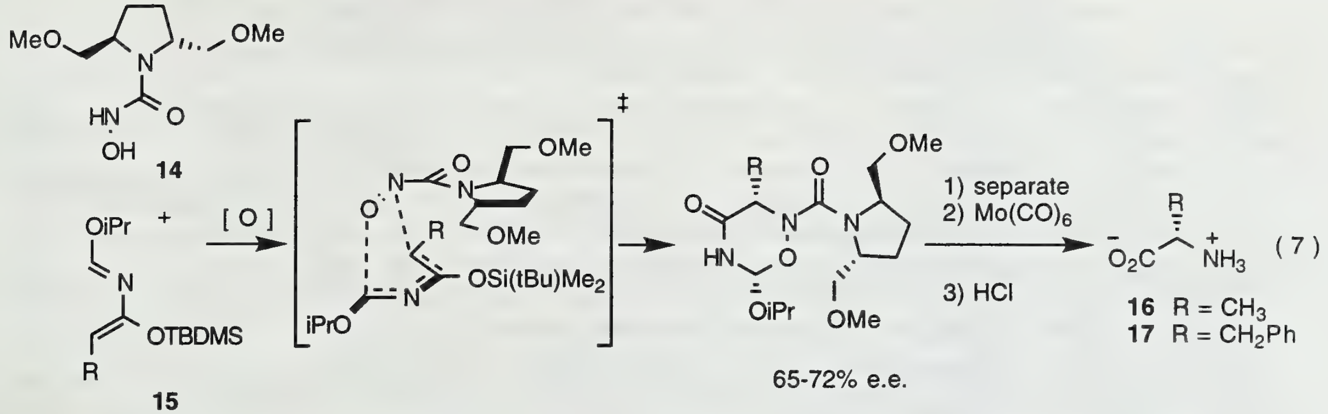
Figure 5. Examples of chiral acyl nitroso compounds

Table I Facial selectivity of hetero Diels-Alder reactions with chiral acyl nitroso compounds (Fig. 5)



Dienophile	n	yield (%)	diastereomer ratio	Reference
7a	2	89	76 : 24 (or 24 : 76)	21
7b	2	83	84 : 16 (or 16 : 84)	21
7c	2	79	82 : 18 (or 18 : 82)	21
8	1	64	13 : 87	20
8	2	73	7 : 93	20
9	1	89	95.5 : 4.5	10
9	2	93	97.5 : 2.5	10
10	1	91-92	99 : 1	16b, 5
10	2	89-94	99 : 1	5, 16b
11	2	81	99 : 1	22
12	2	90	< 1 : > 99	22
13	2	88	99 : 1	5

C_2-symmetric pyrrolidines have been used in the asymmetric synthesis of α-amino acids by Ghosez.[2,28] Corey-Kim oxidation of the corresponding hydroxamic acid **14** produced the nitroso compound **13**, which reacted with 2-azadienes **15** regio- and stereoselectively (eq 7). After the separation of diastereomers, the N-O bond was cleaved with $Mo(CO)_6$ in refluxing aqueous acetonitrile. Hydrolysis produced enantiomerially pure amino acids **16**, **17**.



CONCLUSIONS

The use of stereogenic centers to control the facial selectivity of acyl-nitroso Diels-Alder reactions generates 3,6-dihydro-1,2-oxazines in moderate-to-good yields and moderate-to-high diastereoisomeric excesses, under conditions of kinetic control. In intramolecular and most sterically controlled intermolecular reactions, a syn acyl-nitroso conformation in the transition state is consistant with the observed stereochemistry. In intermolecular reactions where internal hydrogen bonding affects the facial selectivity, either a syn or anti transition state can rationalize the major diastereomer formed. With the availability of various chiral auxiliaries, the asymmetric synthesis of alkaloids and other nitrogen-containing compounds has been achieved.

REFERENCES

(1) (a) Kirby, G.W. *Chem. Soc. Rev.* **1977**, *6*, 1. (b) Weinreb, S. M.; Staib, R. R. *Tetrahedron* **1982**, *38*, 3087. (c) Boger, D.K., Weinreb, S. M. *Hetero Diels-Alder Methodology In Organic Synthesis*, Academic Press, Orlando, 1987. (d) Carruthers, W. *Cycloaddition Reactions in Organic Synthesis*, Pergamon Press, Oxford, 1990. (e) Waldman, H. *Synthesis* **1994**, (6), 535.

(2) Gouveneur, V.; Ghosez, L. *Tetrahedron Lett.* **1991**, *32*, 5349.

(3) Defoin, A.; Schmidlin, C.; Streith, J. *Tetrahedron Lett.* **1984**, *25*, 4515.

(4) Burkholder, T. P.; Fuchs, P. L. *J. Am. Chem. Soc.*, **1990**, *112*, 9601.

(5) (a) Keck, G. E. *Tetrahedron Lett.* **1978**, *48*, 4767. (b) Watanabe, Y.; Iida, H.; Kibayaski, C. *J. Org. Chem.* **1989**, *54*, 4088. (c) Baldwin, J. E.; Bailey, P. D.; Gallagher, G.; Otsuka, M.; Singleton, K. A.; Wallace, P. M.; Prout, K.; Wolf, W. M. *Tetrahedron* **1984,** *40*, 3695.

(6) Keck, G. E.; Romer, D. R. *J. Org. Chem.* **1993**, *58*, 6083.

(7) Corrie, J. E. T.; Kirby, G. W.; Mackinnon, J. W. M. *J. Chem. Soc., Perkin Trans I* **1985**, 883.

(8) (a) Felber, H.; Krezse, G.; Prewo, R.; Vasella, A. *Helv. Chim. Acta* **1986**, *69*, 1137. (b) Hart, H.; Ramaswami, S. K.; Willer, R. *J. Org. Chem.* **1979**, *44*, 1.

(9) (a) Miller, A.; Paterson, T. M.; Proctor, G. *Synlett* **1989**, 32. (b) Dao, L. H.; Dust, J. M.; Mackay, D.; Watson, K. N. *Can. J. Chem.* 1979, 1712.

(10) Kirby, G. W.; McGuigan, H.; McLean, D. *J. Chem. Soc., Perkin Trans. I* **1985**, 1961, and references therein.

(11) (a) Boger, D.L.; Patel, M. *J. Org. Chem.* **1984**, *49*, 4099. (b) Boger, D. L.; Patel, M.; Takusagawa, F. *J. Org. Chem.* **1985**, *50*, 1911.

(12) Defoin, A.; Fritz, H.; Schmidlin, C.; Streith, J. *Helv. Chim. Acta* **1987,** *70*, 554.

(13) (a) Christie, C. C.; Kirby, G. W.; McGuigan, H.; Mackinnon, J. W. M. *J. Chem. Soc., Perkin Trans. I* **1985**, 2469. (b) Keck, G. E.; Yates, J. B. *Tetrahedron Lett.* **1979**, *48*, 4627.

(14) (a) Dubey, S. K.; Knaus, E. E. *J. Org. Chem.* **1985**, *107*, 2080. (b) Kirby, G. W.; Mackinnon, J. W. M. *J. Chem. Soc. Perkin Trans. I* **1985**, 887. (c) Mackay, D.; Dao, L. H.; Dust, J. M. *J. Chem. Soc., Perkin Trans. I* **1980**, 2408.

(15) Carey, F. A.; Sundberg, R. J. *Advanced Organic Chemistry, Part A: Structure and Mechanisms*, third edition, Plenum Press, New York, 1990, and references therein.

(16) Aoyagi, S.; Shishido, Y.; Kibayashi, C. *Tetrahedron Lett.* **1991**, *32*, 4325.

(17) Kirby, G. W.; Nazeer, M. *Tetrahedron Lett.* **1988**, *29*, 6173, and references therein.

(18) Miller, A.; Proctor, G. *Tetrahedron Lett.* **1990**, *31*, 1041.

(19) Defoin, A.; Brouillard-Poichet, A.; Streith, J. *Helv. Chim. Acta* **1992**, *75,* 109.

(20) Gouverneur, V.; Dive, G.; Ghosez, L.*Tetrahedron: Asymmetry* **1991**, *2*, 1173.

(21) Ritter, A. R.; Miller, M. J. *J. Org. Chem.* **1994**, *59*, 4602.

(22) Kirby, G. W.; Nazeer, M. *J. Chem.Soc.,Perkin Trans I* **1993**, 1397.

(23) Defoin, A.; Pires, J.; Tissot, I.; Tschamber, T.; Bur, D.; Zehnder, M.; Streith, J. *Tetrahedron: Asymmetry* **1991**, *2*, 1209.

(24) (a) King, S.B.; Ganem, B. *J. Am. Chem. Soc.* **1991**, *113*, 5089. (b) King, S.B.; Ganem, B. *J. Am. Chem. Soc.* **1994**, *116*, 562.

(25) Brouillard-Poichet, A.; Defoin, A.; Streith, J. *Tetrahedron Lett.* **1989**, *30*(50), 7061.

(26) Cardillo, B.; Galeazzi, R.; Mobbili, G.; Orena, M.; Rossetti, M. *Tetrahedron: Asymmetry* **1994**, *5*, 1535.

(27) Martin, S. F.; Hartmann, M.; Josey, J. *Tetrahedron Lett.* **1992**, *33*, 3583.

(28) Ghosez, L.; Genicot, C.; Gouverneur, V. *Pure & Appl. Chem.* **1992**, *64*, 1849.

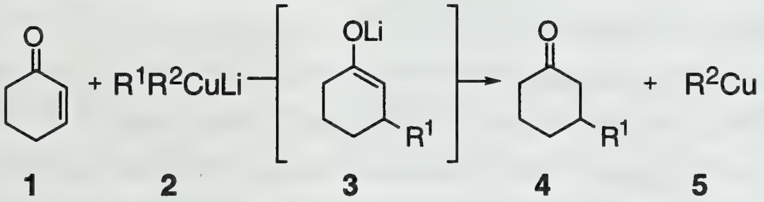
(29) Gouverneur, V.; Ghosez, L. *Tetrahedron: Asymmetry* **1990**, *1*, 363. (b) Defoin, A.; Brouillard-Poichet, A.; Streith, J. *Helv. Chim. Acta* **1991**, *74,* 103.

RECENT ADVANCES IN ORGANOCUPRATE CHEMISTRY: STRUCTURE AND MECHANISM OF CONJUGATE ADDITION

Reported by Robert A. Stavenger October 6, 1994

INTRODUCTION

Organometallic compounds derived from copper have enjoyed a long and rich history in organic chemistry.[1] Copper salts were first used in 1941 to catalyze the 1,4-addition of Grignard reagents to enones.[2] A significant contribution of organocopper reagents to organic synthesis has been in 1,4-conjugate addition reactions, wherein a ligand on copper formally undergoes nucleophilic addition to an electrophilic olefin (eq 1). Understanding the mechanism of this reaction is of utmost importance if the rational design of next-generation reagents is to be expected. Although several points, particularly cyanocuprate structure and the existence of a copper(III) intermediate in the mechanism, are still ambiguous, much has been learned in recent years. This review will discuss the structure of lithium organocuprates and aspects of the mechanism of conjugate addition of these Gilman reagents. Neutral copper alkyl reagents,[1a] cuprates derived from Grignard reagents,[1a,3] and cuprate modification with Lewis acids,[4] or TMSCl,[5] will not be discussed.



(1)

BACKGROUND

Organocopper compounds exist in many stoichiometries (Table I). Lithium organocuprates have been reported to participate in many reactions, such as nucleophilic displacements, bi-aryl couplings, and 1,4-additions to α,β-unsaturated carbonyl compounds. However, these reagents differ in their method of preparation and reactivity towards various substrates. Arriving at the most effective cuprate or cuprate/additive combination for a specific transformation is often a matter of trial and error.

Lower-order (LO) organocuprates (R_2CuLi), also known as Gilman reagents, and higher-order (HO) organocuprates (R_3CuLi_2) have been the most studied and utilized organocopper reagents. Both of these reagents participate in S_N2 processes, including epoxide opening, and in

Copyright © 1994 by Robert A. Stavenger

conjugate addition reactions, though the HO cuprates tend to be less reactive.[1a] Since one ligand of a Gilman reagent is lost as an unreactive copper alkyl byproduct **5**, mixed organocuprates (R^1R^2CuLi) where one ligand cannot transfer have been used. Common non-transferable ligands include 2-thienyl and cyanide.[1] Application of LO and HO cyanocuprates has greatly expanded over the last ten years, owing to their relative stability, ease of preparation, and slightly improved reactivity as compared to Gilman reagents.

Table I. Some Common Cuprate Reagents

cuprate formula	common name
R_2CuLi	lower-order (LO) cuprate, Gilman reagent
R^1R^2CuLi	mixed LO cuprate
R_3Cu_2Li	higher-order (HO) cuprate
$R(CN)CuLi$	LO cyanocuprate
$R_2(CN)CuLi_2$	HO cyanocuprate

REAGENT FORMATION AND STRUCTURE

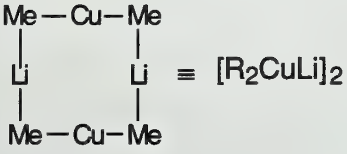
Formation

Lithium organocuprates are typically formed by sequential addition of lithium reagents to a copper salt, such as CuI, in diethyl ether at -78 °C or below. Addition of one equivalent of an organolithium reagent forms LiI and the insoluble copper alkyl species. A second equivalent can then be added, producing the formal "ate" complex or Gilman reagent. HO organocuprates are generated likewise, with a typical ratio of three equivalents lithium reagent for each equivalent copper.[1a] Unfortunately, several ambiguities exist in cuprate formation. For example, formation of lithium dimethylcuprate in diethyl ether followed by dilution with THF or formation of the reagent in a mixture of THF and diethyl ether provides spectroscopically and chemically different entities.[6] These differences often make comparing experimental data challenging, if not impossible.

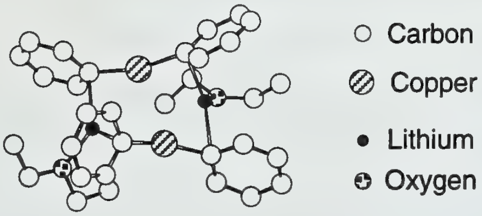
For optimized and reproducible results, a high purity copper salt is required. Copper(I) iodide has been the most common copper source, though more recently copper(I) bromide-dimethyl sulfide complex (CuBr·DMS) and CuCN have become widely used starting materials. Copper(I) iodide can be difficult to purify, and CuBr·DMS has a tendency to decomplex dimethyl sulfide over time or during extended drying.[7] These problems can lead to incomplete reagent formation, or formation of higher aggregates giving misleading results. The reagents derived from CuI and CuBr·DMS are identical;[8] however, when CuCN is used, a different species, namely cyanocuprates, are formed.

Structure

An understanding of reagent structure is a critical first step in elucidating the mechanism of conjugate addition. For 25 years, Gilman reagents have been assigned dimeric structures primarily due to NMR, ebullioscopic, and solution X-ray scattering experiments performed by Pearson on halide free lithium dimethylcuprate (Me_2CuLi) in diethyl ether.[9] A representation of dimeric Me_2CuLi (**6**) is shown in Figure 1. Molecular orbital calculations of Whangbo also predict this square, planar, dimeric structure.[10] Low temperature ^1H NMR analysis has shown that in halide free THF/diethyl ether solutions $[Me_2CuLi]_2$ is in equilibrium with MeLi and a HO cuprate (Me_3CuLi_2).[9b] However, in diethyl ether or in the presence of LiI, only the dimeric form was observed at -78 °C and above. More recently, a solid state structure of a simple, non-aggregated, Gilman reagent, lithium diphenylcuprate $[Ph_2CuLi]_2$ (**7**), was published by Weiss.[11] This structure deviates from the calculated low energy planar conformation; however, the linearity of the R-Cu-R bonds is maintained. Lithium diphenylcuprate has been reported to participate in an equilibrium analogous to $[Me_2CuLi]_2$, although only in DMS.[12] In diethyl ether or THF, the only species detected by ^1H and ^{13}C NMR spectroscopy is assigned as the dimer, $[Ph_2CuLi]_2$.



6



7

Figure 1. Gilman Reagent Dimers

LO cyanocuprates [R(CN)CuLi] have been examined by low temperature ^1H and ^{13}C NMR spectroscopy and do not appear to undergo the equilibrium processes that Gilman reagents participate in. Two bond ^{13}C-^{13}C coupling constants of Me(CN)CuLi (99% enriched, ^{13}CN and $^{13}CH_3$) at -110 °C in THF suggest a single discrete reagent with a linear array of CH_3-Cu-CN.[13] It is believed that the lack of coupling at higher temperatures is due to an exchange process involving Li.[13] However, if HMPA (10 vol. %) is added to the solution, coupling can be seen at temperatures as high as -78 °C. The complexation of Li by HMPA apparently interferes with this exchange, and therefore coupling is observed at higher temperatures.

Similar experiments performed on HO cyanocuprates raise additional questions. Bertz did not observe coupling in the ^{13}C NMR spectrum of $Me_2(CN)CuLi_2$ (99% enriched ^{13}CN and $^{13}CH_3$, -100 °C) in THF with or without HMPA.[14] Also, the ^{13}C resonances of cuprates (dimethyl, diethyl, and diphenyl) derived from CuI and CuCN were very similar, suggesting that higher order cyanocuprates do not exist as independent species in THF, but are Gilman reagents with LiCN complexed within the dimeric cluster $[R_2CuLi{\cdot}LiCN]_2$. This interpretation has been rebutted by Lipshutz, who does not detect free LiCN in THF or DMS/HMPA solution by IR analysis.[15] Furthermore, upon addition of LiCN to Me_2CuLi in THF or DMS, ^1H NMR signals for $[Me_2CuLi]_2$ disappear and the signal for the higher-order cyanocuprate species appeared. This leads him to conclude that cyanocuprates do exist with cyanide bound to copper. The absence of chemical shift difference between the cuprates derived from CuCN and CuI is presumably a solvent dependent phenomenon. Possible explanations include a lack of difference in chemical shift for these species in THF, or cyanocuprates simply do not exist in THF.

Contradictory results are given by Oehlschlager.[16] His experiments, IR (-30 °C) and ^{13}C NMR (-78 °C) spectroscopy, show that in THF/HMPA the dominant complexes are the Gilman reagent and free LiCN. However, in THF or DMS, there is no dissociation, and the HO cyanocuprates probably exist as aggregates. Ab initio calculations suggest that the empirical species $R_2(CN)CuLi_2$ should not exist as tricoordinate copper(I), but should be represented as a Gilman reagent complex with LiCN or LO cyanocuprate complexed with MeLi.[17] It was also suggested that the LiCN acts to equilibrate several unidentified copper species, leading to their differential reactivity as compared to Gilman reagents.[17] Experiments by Knochel also deviate from the Lipshutz assignment of a direct CN-Cu contact. Extended X-ray absorption fine structure (EXAFS) spectroscopy studies show that approximately 90% of the copper is not complexed to cyanide in THF.[18] The results suggest that different forms of the "cyanocuprate" may exist in different solvents. This is not completely surprising, as the difference between the reagents labeled $R_2CuLi{\cdot}LiCN$ and $R_2(CN)CuLi_2$ may be a small difference in the organization of the aggregate. Unfortunately, few of these experiments were run in the same solvent, even "THF" experiments often contain varying amounts of diethyl ether from the MeLi addition, making direct comparison of the results extremely difficult.

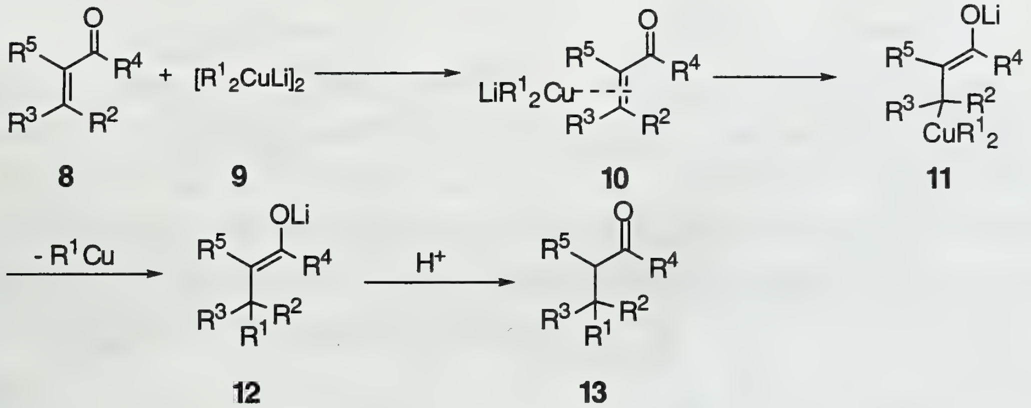
MECHANISM

Most mechanistic studies of cuprate conjugate addition involve Gilman reagents, usually Me_2CuLi, and it is generally assumed that cyanocuprates follow the same basic pathway. However, it may be naive to make these assumptions with such sensitive reagents, especially without solid structural information The process of conjugate addition via organocuprates has three main steps (Scheme I); (1) association of enone and cuprate, (2) oxidative addition of cuprate into the alkene, and (3) reductive elimination to give the enolate product . Unfortunately, the reductive elimination step has not been studied in any detail. This is primarily owing to a lack of evidence for the existance of the precursor copper(III) adduct, **11**.

Olefin Coordination

Stopped flow kinetic studies by Smith[19] have indicated the existence of an intermediate in the reaction of $Me_2CuLi{\cdot}LiI$ with several enones. This intermediate was presumed to be a Li^+ coordinated form of the enone based on UV data. However, it was unclear whether free LiI, or Li^+ in the cuprate cluster was acting on the carbonyl oxygen. Though there was no examination of coordination of the copper and the enone, it seems likely that d,π* coordination was taking place at the same time as Li^+ coordination.

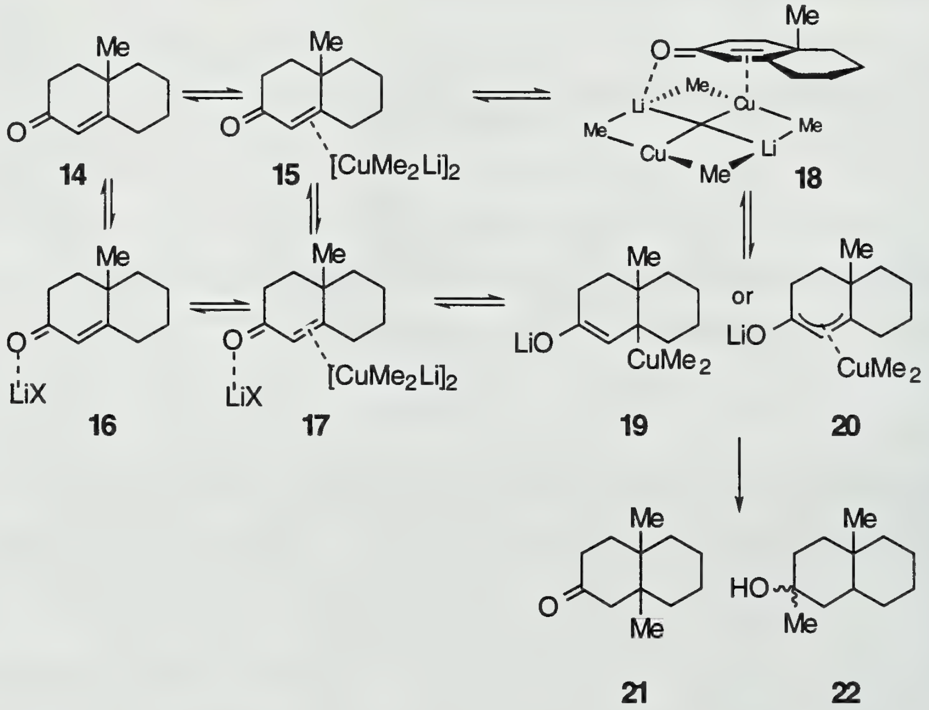
Scheme I



The complexation of Li^+ to the carbonyl group of enones, with or without associated d,π* copper enone interaction, has also been detected by variable temperature NMR spectroscopy. In the reaction of octalone **14** with two equivalents of halide free lithium dimethylcuprate dimer in diethyl ether (Scheme II), four different copper-enone complexes can be observed by ^{13}C NMR at -78 °C.[20] These were assigned as the exo and endo complexes of enone with the monomeric and the dimeric cuprate. When one equivalent of dimer was added to **14**, several intermediates were detected including complexes **15** and **16**. Two other complexes with both lithium and copper coordination were also observed and assigned as exo and endo **17**. However, later work of Ullenius has suggested that the nominally flat dimer approaches the π-system of the enone (as in **18**), coordinating to both the olefin and the carbonyl group simultaneously.[21] In the ^{13}C NMR spectrum of the complex (presumably **18**) the resonance for the β-carbon of the enone is shifted 87 ppm upfield, while that for the α-carbon is only shifted 43 ppm upfield. This suggests stronger Cu-C_β interaction, possibly leading toward the β-copper(III) intermediate **19**. In this study, while the 1,2- product was formed immediately, a delay was observed in the formation of the 1,4-product. This eliminates the possibility of a π-allyl copper intermediate **20**, as it would be expected to form both 1,2- and 1,4-products simultaneously. The 1,2-product **22** was argued to have arisen from the addition of residual MeLi, whereas the 1,4 product **21** arises from a d,π* copper enone complex followed by oxidative addition/reductive elimination.

Further studies come from the work of Ullenius on the complexation of ^{13}C- enriched cinnamate esters.[21] ^{13}C NMR analysis suggests that Me_2CuLi formed a π-complex with *t*-butyl cinnamate in toluene/THF mixtures. The complex was relatively stable below -40 °C, while at higher temperatures formation of enolate was seen. Ullenius assigns the complex as the dimer-enoate complex analogous to **18**, however the presence of signals at -6.8, -8.4, and -9.3 ppm, all assigned to complexed Me_2CuLi, implies that more than one complex was formed.

Scheme II

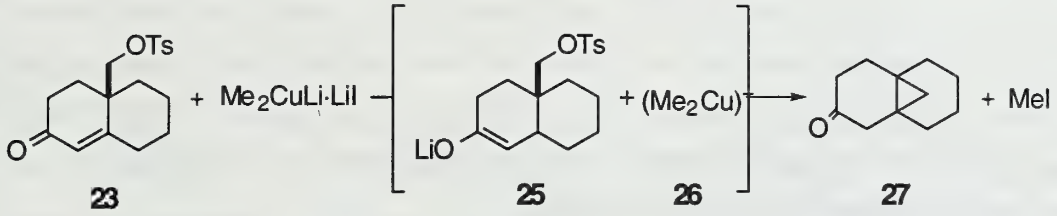


Oxidative Addition

The formal oxidative addition producing the copper(III) intermediate **11** (Scheme I) is not nearly as well studied or understood as the d,π* complex formation. Although the oxidative addition has been claimed to be a direct nucleophilic addition or a reversible two-electron process, other results point to a stepwise single electron transfer (SET) oxidative addition. House and co-workers have long presumed that the reaction proceeds by a SET mechanism, based on the observation that cuprate reactivity varies with enone reduction potential.[22] In addition, easily reduced enones tend to give reduction products, presumably from transfer of a second electron to the anion radical forming the dianion.

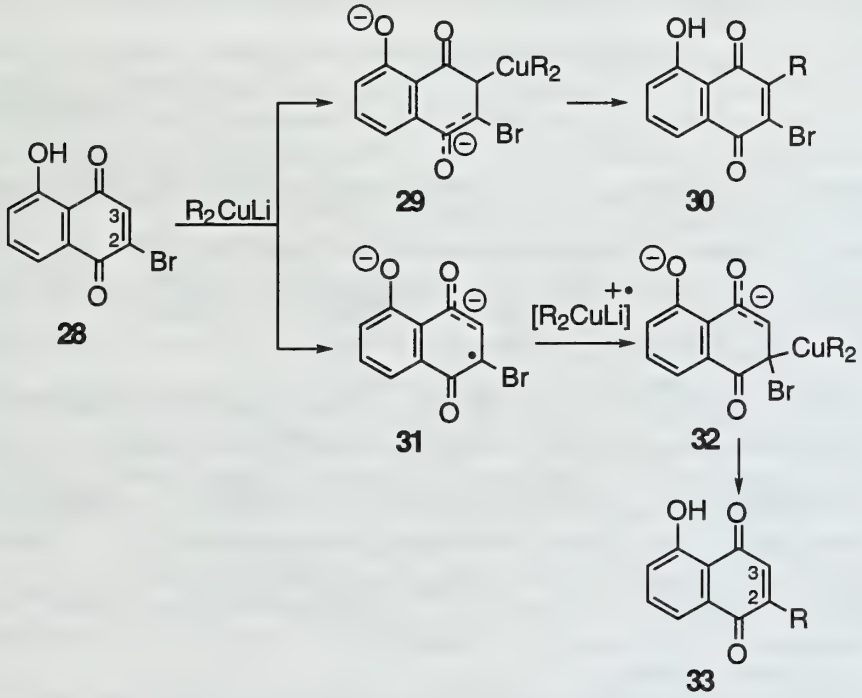
Addition of Me_2CuLi to tosylate **23** provides the cyclopropyl product **27** and MeI in 87% and 95% yield, respectively (Scheme III).[23] This was interpreted as a two-electron transfer, creating a formal negative charge on the β-position of the enone which then displaced the tosylate. The MeI is believed to arise from attack of I^- on the oxidized copper species "Me_2Cu^+" (**26**). House refutes this theory by stating that the cuprate is not a strong enough reductant to form the enolate without some bonding participation of the copper, and that it is a copper assisted nucleophilic substitution.

Scheme III



Recently more definitive proof that SET is operating in the oxidative addition step has been reported.[24] Naphthoquinone **28** (Scheme IV) is biased toward nucleophilic attack at the 3-position and radical stabilization at the 2-position. Thus, if the addition is nucleophilic, attack should occur at the least hindered site (**29**), whereas if it is a single electron process, the coupling should occur at the site of highest spin density (**31** and **32**). Their results show complete selectivity for the products of the presumed SET process (**33**).

Scheme IV



CONCLUSION

Organocopper compounds are very useful reagents for organic synthesis. Unfortunately, a unified theory on their structure and mechanism of action does not exist, despite a number of elegant studies. The primary reason these reagents are not more fully understood is their subtle dependence on solvent and possible aggregation state Without consistent reaction conditions it may be irrelevant to make any claims based on a comparison of data from two or more studies.

Although a reasonable mechanism for Gilman reagent 1,4-conjugate addition has been proposed, several issues remain, notably lack of proof of a β-copper(III) intermediate and whether it's formation is the rate limiting step. In addition, there is little evidence to show a direct correlation between the structure and mechanism of cyanocuprate (or HO cuprate) reactions; although, they are presumed to follow the same pathway. The experiments that have been performed are sound, though a better understanding of cuprate structure and reactivity would come from studies where dependence on solvent and organolithium source are each treated independently. Only then can a full picture organocuprate chemistry be realized.

References

1. For recent reviews see: (a) Lipshutz, B. H.; Sengupta, S. *Org. React.* **1992**, *41*, 135. (b) Duval, D.; Geribaldi, S. In *The Chemistry of Enones Part I*; Patai, S.; Rappoport, Z., Eds.; John Wiley & Sons: New York, 1989.
2. Kharasch, M. S.; Tawney, P. O. *J. Am. Chem. Soc.* **1941,** *63*, 2308.
3. (a) Lipshutz, B. H.; Parker, D. A.; Nguyen, S. L.; McCarthy, K. E.; Barton, J. C.; Whitney, S. E.; Kotsuki, H. *Tetrahedron* **1986**, *42*, 2873. (b) Bertz, S. H.; Gibson, C. P.; Dabbagh, G. *Organometallics* **1988**, *7*, 227.
4. Yamamoto, Y. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 947.
5. For a leading reference see: Lipshutz, B. H.; Dimock, S. H.; James, B. *J. Am. Chem. Soc.* **1993**, *115*, 9283.
6. Lipshutz, B. H.; Kozlowski, J. A.; Breneman, C. M. *J. Am. Chem. Soc.* **1985**, *107*, 3197.
7. Bertz, S. H.; Gibson, C. P.; Dabbagh, G. *Tetrahedron Lett.* **1987**, *28*, 4251.
8. Lipshutz, B. H.; Whitney, S.; Kozlowski, J. A.; Breneman, C. M. *Tetrahedron Lett.* **1986**, *27*, 4273.
9. (a) Pearson, R. G.; Gregory, C. D. *J. Am. Chem. Soc.* **1976**, *98*, 4098. See also (b) Ashby, E. C.; Watkins, J. J. *J. Am. Chem. Soc.* **1977**, *99*, 5312.
10. Stewart, K. R.; Lever, J. R.; Whangbo, M.-H. *J. Org. Chem.* **1982**, *47*, 1472.
11. Lorenzen, N. P.; Weiss, E. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 300.
12. Bertz, S. H.; Dabbagh, G. *J. Am. Chem. Soc.* **1988**, *110*, 3668.
13. Bertz, S. H. *J. Am. Chem. Soc.* **1991**, *113*, 5470.
14. Bertz, S. H. *J. Am. Chem. Soc.* **1990**, *112*, 4031.
15. Lipshutz, B. H.; Sharma, S.; Ellsworth, E. L. *J. Am. Chem. Soc.* **1990**, *112*, 4031.
16. Singer, R. D.; Oehlschlager, A. C. *J. Org. Chem.* **1992**, *57*, 2192.
17. Snyder, J. P.; Spangler, D. P.; Behling, J. R. *J. Org. Chem.* **1994**, *59*, 2665.
18. Stemmler, T.; Penner-Hahn, J. E.; Knochel, P. *J. Am. Chem. Soc.* **1993**, *115*, 348.
19. Kraus, S. R.; Smith, S. G. *J. Am. Chem. Soc.* **1981**, *103*, 141.
20. Bertz, S. H.; Smith, R. A. J. *J. Am. Chem. Soc.* **1989**, *111*, 8276.
21. (a) Hallnemo, G.; Olsson, T.; Ullenius, C. *J. Organomet. Chem.* **1984**, *265*, C22. see also: Christenson, B.; Ullenius, C.; Hakansson, M.; Jagner, S. *Tetrahedron* **1992**, *48*, 3623. and references therein.
22. For a review of House's work on SET in organocuprates see: House, H. O.; *Acc. Chem. Res.* **1976**, *9*, 59.
23. Hannah, D. J.; Smith, R. A. J. *Tetrahedron Lett.* **1975**, *24*, 187.
24. Wigal, C. T.; Grunwell, J. R.; Hershberger, J. *J. Org. Chem.* **1991**, *56*, 3759.

PREPARATION, STEREOSELECTIVE TRANSFORMATIONS, AND APPLICATION OF 6-HYDROXY-3-PYRANONES IN THE SYNTHESIS OF POLYOXYGENATED COMPOUNDS

Reported by Bryan C. Duffy October 17, 1994

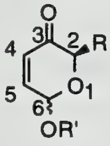
INTRODUCTION

Many complex, oxygenated natural products, such as carbohydrates, *C*-glycosides, hydropyrans, macrolides, and ionophores have been synthesized by stereoselective transformations of substituted 6-hydroxy-3-pyranones.[1] These oxygen heterocycles have become attractive intermediates in multi-step syntheses as a result of improved methods for their preparation from the versatile 2-furylcarbinols. Recent syntheses of monocrotaline and (+)-KDO are founded on stereoselective transformations to 6-hydroxy-3-pyranone derived intermediates.[2,3] The preparation of 6-hydroxy-3-pyranones by oxidation of 2-furylcarbinols, stereoselective transformations, and examples in natural product syntheses involving 6-hydroxy-3-pyranones will be discussed in this seminar.

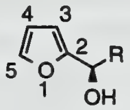
OXIDATIVE TRANSFORMATION OF 2-FURYLCARBINOLS INTO 6-HYDROXY-3-PYRANONES

General Considerations

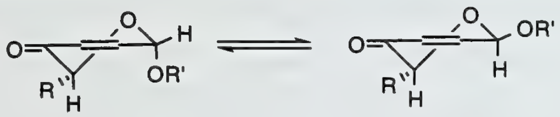
Substituted 6-hydroxy-3-pyranones, **1a**, are most frequently prepared by oxidation of substituted 2-furylcarbinols, **2**.[4] Several different oxidation reagents and procedures have been developed for this purpose.[5] Although the 2-furylcarbinol stereochemistry is conserved during oxidation, equilibration at the new stereogenic center, C-6, results in the formation of a thermodynamic mixture of isomers, **3a** and **4a**. For R = CH_3 and R´ = H, the *trans-* isomer is favored, due to anomeric stabilization. For R´ = alkyl or acyl, mixtures of **3b** and **4b** usually result. Conformers which place the largest C-2 substituent in an equatorial position are favored to reduce axial interactions. The degree of anomeric stabilization is dependent on the electron withdrawing ability of R´, solvent polarity, and ring substituents. Diastereomer structural assignments are based on ^{1}H-NMR determination of H-4 / H-6 and H-5 / H-6 coupling frequencies.[5b-e]



1



2



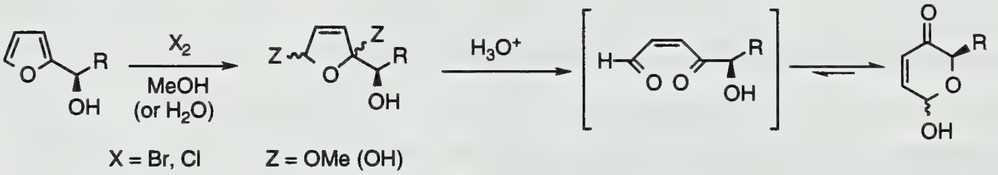
3 a,b **4 a,b**

a: R' = H
b: R' = alkyl or acyl

Copyright © 1994 by Bryan C. Duffy

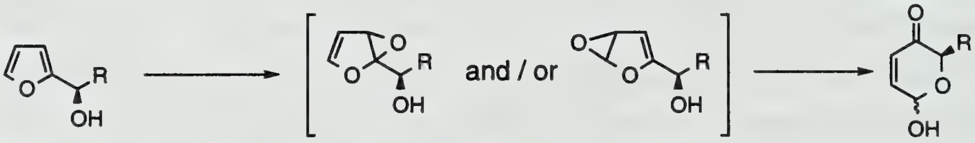
Oxidative Methods

Bromination in methanolic THF directly forms the bromo ether adduct, which undergoes solvolytic conversion to the isolable 2,5-dimethoxyfuran. Acidic hydrolysis of this dimethyl ether produces an unstable, oxoenal isomer, which rapidly cyclizes to the more stable 6-hydroxy-3-pyranone (Equation 1).[4] In aqueous THF, the bromohydrin adduct undergoes hydrolytic ring opening to the same acyclic isomer with subsequent cyclization. Anodic oxidation of 2-furylcarbinols is a much milder method that provides the same 2,5-dimethoxyfuran intermediate as bromination in methanol.[5a] The *N*-bromosuccinimide (NBS) oxidation method uses a colorometric titration of the 2-furylcarbinols in an aqueous THF mixture at 0 °C to achieve better stoichiometric control and selectivity.[5b-e]



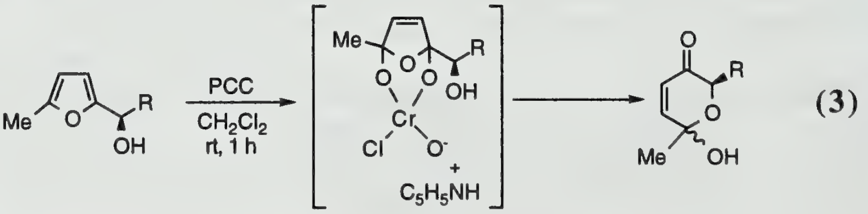
(1)

Peracids offer an alternative reagent for 2-furylcarbinol oxidation in the preparation of 6-hydroxy-3-pyranones (Equation 2). Peracid epoxidation of either furan ring double bond is followed by fragmentation to the same oxoenal intermediate, which rapidly undergoes ring closure to the substituted 6-hydroxy-3-pyranone. Peracetic acid and *meta*-chloroperbenzoic acid (*m*-CPBA) are the two most commonly used peracids for this transformation.[6a,b] However, vanadyl acetylacetonate ($VO(acac)_2$) / *t*-butyl hydroperoxide (t-BuOOH) gives higher overall yields in some cases.[6c]



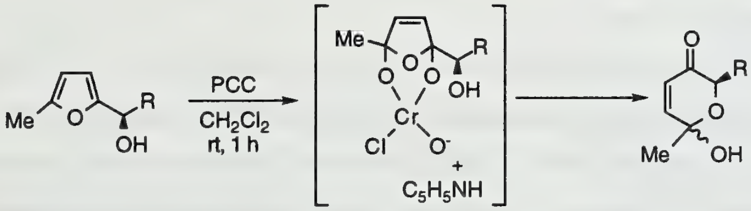
(2)

The same oxidation can also be accomplished with pyridinium chlorochromate (PCC). Although the PCC method was originally developed for the oxidation of furans to α,β-unsaturated γ-dicarbonyl compounds, it provides an easy one-step oxidation to the desired 6-hydroxy-3-pyranones.[7a] Piancatelli has proposed the bridged chlorochromate adduct in Equation 3 as a reaction intermediate.[7b]



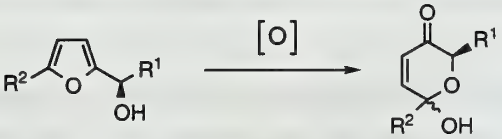
(3)

Singlet oxygen is another well studied, oxidative reagent used for converting substituted furans to 6-hydroxy-3-pyranones.[8a] Singlet oxygen oxidation of furans produces the corresponding 5-hydroxy-2-furanone, by disproportionation of the endoperoxide adduct upon warming; however, immediate reduction of the unstable endoperoxide with mild reducing agents, such as triphenylphosphine or dimethyl sulfide, affords the 6-hydroxy-3-pyranones (Equation 4).[8b,c] Typical reaction conditions involve the irradiation of the 2-furylcarbinol with oxygen in a CH_2Cl_2 / methanol solvent system at -60 °C with tungsten bromide lamps.[8d,e] Typical yields for the preceding oxidative methods on a variety of substituted 2-furylcarbinols are compared in Table I.



(3)

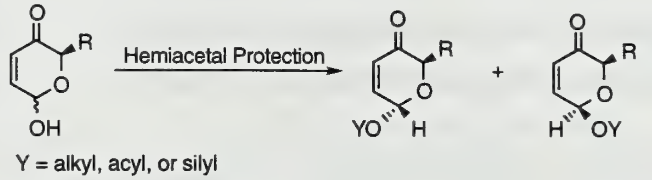
Table I Comparison of Various Reagents for Oxidation of 2-Furylcarbinols to 3-Pyranones



R^1	R^2	Method	Yield (%)	Reference
H	H	*m*-CPBA	48	6b
H	H	Anodic Oxidation	37	8e
H	H	$VO(acac)_2$ / t-BuOOH	45	6c
CH_3	H	NBS	65	5
CH_3	H	Anodic Oxidation	60	8e
CH_3	H	$^1O_2$ / Me_2S	90	8b
CH_3	H	$VO(acac)_2$ / t-BuOOH	65	6b
CH_3CH_2	H	Anodic Oxidation	73	8e
nBu	H	$^1O_2$ / Me_2S	65	8d
CH_3	CH_3	PCC	>90	7a
CH_3	CH_3	$^1O_2$ / Me_2S	89	8a

MODIFICATION OF THE 6-OH POSITION

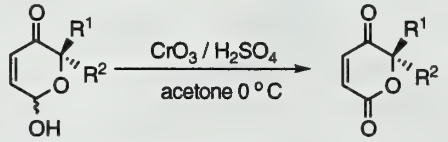
The 6-position may be protected against anomeric equilibration or activated for substitution by acylation, alkylation, or silation. These stable derivatives may be separated as individual diastereomers for further synthetic transformations. The use of a Lewis acid catalyst allows synthesis of 6-*O*-alkylated compounds in good yield via substitution of the benzoate glycoside.[9a] The use of zinc(II) chloride etherate in 1,2-dichloroethane as the Lewis acid gives superior yields to the previously used tin(IV) chloride substitution procedures.[5b] Alternative procedures of hemiacetal protection include the reaction of the 6-hydroxy-3-pyranone with trialkyl orthoformates in the presence of Lewis acids, alkyl halides in the presence of silver(I) oxide, silyl halides in the presence of silver(I) oxide, or carboxylic anhydride with sodium acetate (Equation 5).[4,5d,9b,c]



(5)

Georgiadis has developed alternative methods for the synthesis of the 6-acyloxy and 6-alkoxy compounds from 6-hydroxy-3-pyranones.[9d] The 6-*O*-acylated compounds are formed by the addition of 1,3-dicyclohexylcarbodiimide (DCC) to a stirred solution of the protecting group as a carboxylic acid, substituted 6-hydroxy-3-pyranone, and 4-dimethylaminopyridine (DMAP) in THF at 0 °C. These methods avoid the use of strong acids or bases and usually produce the *trans*-isomer in good yield.

Oxidation of the hemiacetal in 6-hydroxy-3-pyranones is most commonly carried out with chromic acid and sulfuric acid (Jones reagent) in acetone at 0 °C to give γ-oxo-lactones (Equation 6).[9b,e] The lactones are synthetically useful for further transformations, including the ring opening to 1,4-oxo compounds.[9b]



(6)

MODIFICATION AT THE DOUBLE BOND

Dihydroxylation By Osmium Tetroxide

Dihydroxylation by osmium tetroxide at the double bonds of 3-pyranones and 3-pyranols has been used in the synthesis of carbohydrates and oxygenated natural products. The use of osmium tetroxide to form the *cis*- diol on the less hindered face of the ring is known to give excellent selectivity and good yields. Osmylation is known to occur at the face opposite of allylic hydroxyl and ether groups. Steric approach control and hydroxyl direction allow accurate predictions of osmylation stereochemistry. Osmylations of the electron deficient double bond in an α,β-enone are generally slow.[10a] If both a carbonyl reduction and *cis*- dihydroxylation are required, the carbonyl reduction is typically performed first to increase reactivity and improve stereoselectivity of the osmylation.[10b]

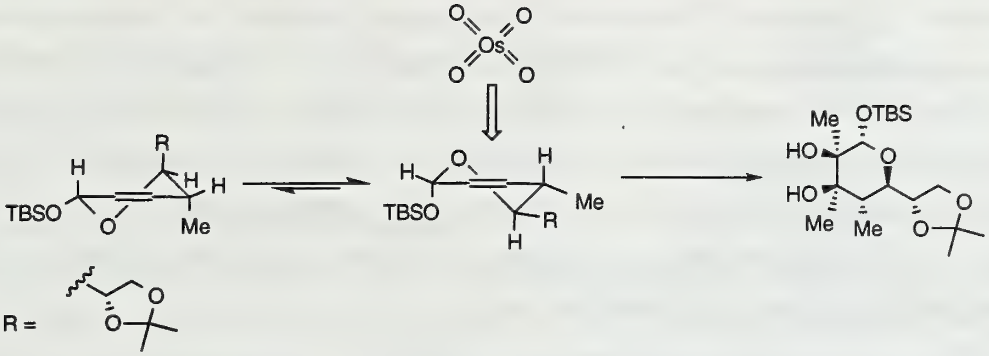
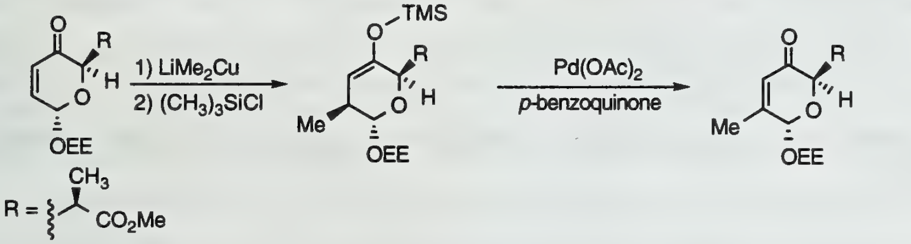


Figure 1. Osmylation Facial Selectivity (Note: olefinic methyls removed for clarity)

Conjugate Additions

Conjugate addition of lithium dimethylcuprate at the C-5 site of protected 6-hydroxy-3-pyranones proceeds selectively on the face opposite to the C-6 oxygen substituent.[11a] In Martin's synthesis of (+)-Prelog-Djerassi lactone, a conjugate lithium dimethylcuprate addition occurred stereoselectively on the β-face of the ethoxyethyl protected 6-hydroxy-3-pyranone (Equation 7).[11b] A Ito-Saegusa oxidation of the resulting trimethylsilyl enolether regenerated the α,β–enone.[11c]



(7)

Stereochemical predictions of conjugate additions to protected 6-hydroxy-3-pyranones can be made based on the steric hindrance of approach from both sides of the ring (Figure 2). The most stable conformer is predicted based on the minimization of diaxial interactions. Placement of the R group in an equatorial position assists in conformational stability. Analysis of steric hindrance on each side of the ring in both conformers, leads to prediction of conjugate addition on the face opposite 6-*O* substituent.

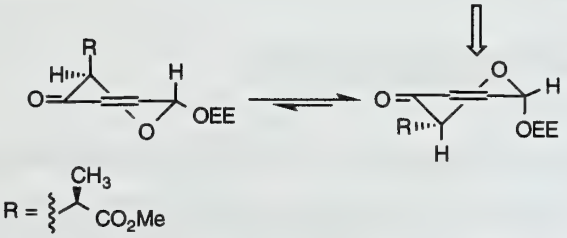
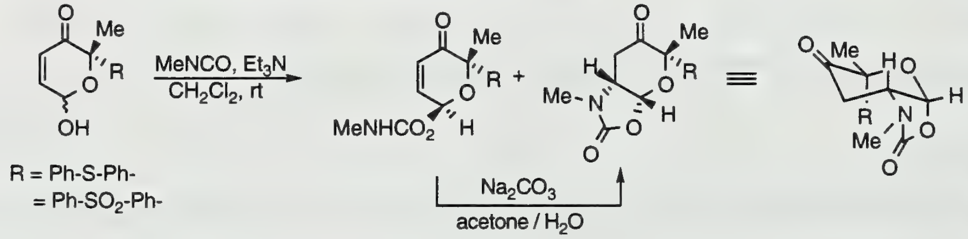


Figure 2. Most Stable Conformer and Least Hindered Face (Note: olefinic hydrogens removed for clarity)

Formation of substituted 5*H*-pyrano-[3,2-*d*]oxazole-2,6-diones by stereoselective ring closure opened a convenient route to 2-aminosugar precursors.[11d] Treatment of substituted 3-pyranones with methyl isocyanate and triethylamine at room temperature leads to the carbamate and the bicyclic 3-pyranone adduct (Equation 8). Only the *trans-* isomer (C-2 methyl to 6-*O*) forms a stable, bicyclic oxazolidinone owing to the steric interaction from the R group in forming the *cis-* isomer adduct. Under basic conditions, the acyclic *cis-* isomer can be equilibrated to the *trans-* isomer, which rapidly cyclizes to the oxazolidinone.



(8)

FUNCTIONALIZATION AT THE CARBONYL CARBON

Reduction

Reduction of the carbonyl in 6-*O*-protected 3-pyranones is mainly controlled by steric interactions of the approaching hydride source with the ring substituents. Bulky hydride donors attack from the side of least steric interaction, which favors axial alcohol formation. The standard reduction reagents of $NaBH_4$ / $CeCl_3$ and potassium tri-*sec*-butylborohydride (K-Selectride®) provide the equatorial and axial alcohols respectively in high yield with usually >8:1 stereoselectivity.[2,3,12]

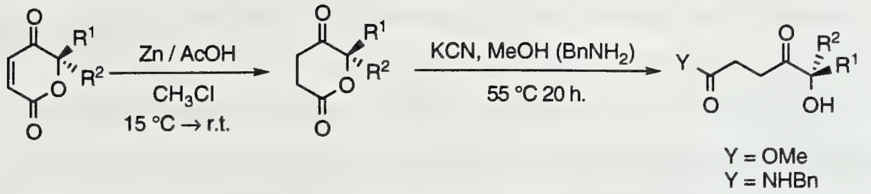
Olefination

The Wittig reactions with ylides formed from either triphenylphosphonium bromide / potassium t-butoxide or triphenylphosphonium iodide / n-butyllithium give moderate yields of the exo-methylene product.[2,3] While good E, Z selectivity for functionalized ylides has not been realized for the Horner-Emmons olefination, with or without the Stille modification, the Peterson olefination has been effective in one case of carboxymethylenation, affording the (E)-α,β-unsaturated ester in >7:1 ratio.[13]

3-PYRANONE RING TRANSFORMATIONS

Ring Opening

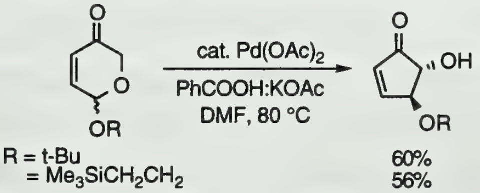
Ring opening of γ-oxo-δ-lactones with potassium cyanide in the presence of an alcohol or amine is a useful route to δ-hydroxy-γ-oxo-esters and amides (Equation 9).[9b] The keto-lactones are prepared by Jones oxidation of 6-hydroxy-3-pyranones followed by double bond reduction with zinc powder in acetic acid. Treatment of a γ-oxo-δ-lactone with methanolic potassium cyanide affords the methyl ester, while addition of benzylamine in the absence of methanol gives the related benzylamide.



(9)

Ring Contraction To Cyclopentenones

The catalytic aldol contraction of a 6-*O*-alkyl protected 3-pyranone ring with palladium(II) acetate in buffered anhydrous DMF provides a rapid route to *trans*-4,5-dioxygenated-2-cyclopentenones (Equation 10).[9a] Protection of the hemiacetal with the tert-butyl or 2-(trimethysilyl)ethyl ethers improves product yields, by reduced alkoxide elimination. The reaction fails in the presence of C-2 substitution, limiting the choice of suitable substrates.[14]



(10)

Spiroketal Formation

The spiroketal moiety is commonly found in many classes of natural products, such as pheromones and antibiotics. A possible advantage of the 3-pyranone spiroketalization over acyclic reaction variants is the increased number of internal interactions that could be used for stereoselective modifications. The placement of alkyl substituents in the C-2 position of the 6-hydroxy-3-pyranone allows for thermodynamic equilibration of the spiroketal conformation (Figure 2). The equilibration minimizes the axial interactions while also allowing "dual anomeric stabilization at carbon 2".[12]

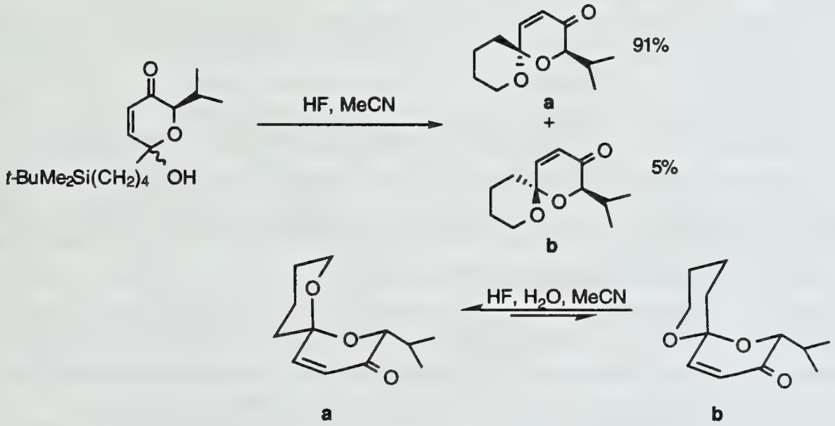


Figure 2. Thermodynamically Controlled Spiroketalization

CONCLUSIONS

Substituted 6-hydroxy-3-pyranones are useful intermediates for stereoselective synthesis of many oxygenated compounds. These heterocyclic cyclohexenones are conveniently prepared by oxidation of 2-furylcarbinols. Osmylation and conjugate addition are predicted to occur on the face opposite the 6-*O* substituent. Multiple sites of differing reactivity allow specific introduction of functionality to the 3-pyranone structure. Some examples of natural product syntheses using these methods for stereoselective functionalization of 6-hydroxy-3-pyranones include maltol, (+)-KDO, erythronolide B, tirandamycin B, and (+)-Prelog-Djerassi lactone.[11a,15]

REFERENCES

(1) Martin, S.F.; Gluchowski, C.; Campbell, C.L.; Chapman, R.C. *Tetrahedron* **1988**, *44*, 3171.

(2) Honda, T.; Tomitsuka, K.; Tsubuki, M. *J. Org. Chem.* **1993**, *58*, 4274.

(3) Martin, S. F.; Zinke, P.W. *J. Org. Chem.* **1991**, *56*, 6600.

(4) Achmatowicz, O.; Bukowski, P.; Szechner, B.; Zwierzchowska, Z.; Zamojski, A. *Tetrahedron* **1971**, *27*, 1973, and references therein.

(5) (a) Nedenskov, P.; Elming, N.; Nielson, J.T.; Clauson-Kaas, N. *Acta Chem. Scand.* **1955**, *9*, 17. (b) Georgiadis, M.P.; Couladouros, E.A. *J. Org. Chem.* **1986**, *51*, 2725, and references therein. (c) Grynkiewicz, G.; Barszczak, B.; Zamojski, A. *Synthesis* **1979**, 364. (d) Jaworska, A.; Zamojski, A. *Carbohydr. Res.* **1984**, *126*, 191. (e) Achmatowicz, A.; Bielski, R. *Carbohydr. Res.* **1977**, *55*, 165.

(6) (a) Laliberté, R.; Médawar, G.; Lefebvre, Y. *J. Med. Chem.* **1973**, *16*, 1084. (b) Lefebvre, Y. *Tetrahedron Lett.* **1972**, 133. (c) Ho, T.L.; Sapp, S. G. *Synth. Comm.* **1983**, *13*, 207.

(7) (a) Piancatelli, G.; Scettri, A.; D'Auria, M. *Tetrahedron Lett.* **1977**, *25*, 2199. (b) Piancatelli, G.; Scettri, A.; D'Auria, M. *Tetrahedron* **1980**, *36*, 661.

(8) (a) Foote, C.S.; Wuesthoff, M.T.; Wexler, S.; Burstain, I.G.; Denny, R.; Schenck, G.O.; Schulte-Elte, K.J. *Tetrahedron* **1967**, *23*, 2583. (b) Kuo, Y.H.; Shih, K.S. *Heterocycles* **1990**, *31*, 1941. (c) Sammes, P.G.; Street, L.J. *J. Chem. Soc. Chem. Commun.* **1983**, 666. (d) Sammes, P.G.; Street, L.J.; Whitby, R.J. *J. Chem. Soc. Pekin Trans. I* **1986**, 281. (d) Lee, G.C.M.; Syage, E.T.; Harcourt, D.A.; Holmes, J.M.; Garst, M.E. *J. Org. Chem.* **1991**, *56*, 7007. (e) Shono, T.; Matsumura, Y. *Tetrahedron Lett.* **1976**, *17*, 1363.

(9) (a) Mucha, B.; Hoffmann, H.M.R. *Tetrahedron Lett.* **1989** *30*, 4489. (b) Georgiadis, M.P.; Apostolopoulos, C.D.; Haroutounian, S.A. *J. Heterocycl. Chem.* **1991**, *28*, 599. (c) Weeks, P.D.; Kuhla, D.E.; Allingham, R.P.; Watson, H.A.; Wlodecki, B. *Carbohydr. Res.* **1977**, *56*, 195. (d) Constantinou-Kokotou, V.; Kokotos, G.; Georgiadis, M.P. *Liebigs Ann. Chem.* **1991**, 151, and references therein. (e) Bowers, A.; Halsall, T.G.; Jones, E.R.H.; Lemin, A.J. *J. Chem. Soc.* **1953**, 590.

(10) (a) Achmatowicz, O.; Grynkiewicz, G. *Carbohydr Res.* **1977**, *54*, 193. (b) Dziewiszek, K.; Chmielewski, M.; Zamojski, A. *Carbohydr. Res.* **1982**, *104*, C1.

(11) (a) Plaumann, D.E.; Fitzsimmons, B.J.; Ritchie, B.M.; Fraser-Reid, B. *J. Org. Chem.* **1982**, *47*, 941. (b) Martin, S.F.; Guinn, D.E. *J. Org. Chem.* **1987**, *52*, 5588. (c) Yoshihiko, I.; Hirao, T.; Saegusa, T. *J. Org. Chem.* **1978**, *43*, 1011. (d) Georgiadis, M.P.; Couladouros, E.A. *J. Heterocycl. Chem.* **1990**, *27*, 1757.

(12) DeShong, P.; Waltermire, R.E.; Ammon, H.L. *J. Am. Chem. Soc.* **1988**, *110*,1901.

(13) (a) Bennett, M.; Gill, B.; Pattenden, G.; Shuker, A.J.; Stapleton, A. *J. Chem. Soc. Perkin Trans. I* **1991**, 929. (b) Stille, W.C.; Gennari, C. *Tetrahedron Lett.* **1983**, *24*, 4405.

(14) Kolb, H.C.; Hoffmann, H.M.R. *Tetrahedron* **1990**, *46*, 5127.

(15) (a) Weeks, P.D.; Brennan, T.M.; Brannegan, D.P.; Kuhla, D.E.; Elliot, M.L.; Watson, H.A.; Wlodecki, B.; Breitenbach, R. *J. Org. Chem.* **1980**, *45*, 1109. (b) Martin, S.F.; Zinke, P.W. *J. Org. Chem.* **1991**, *56*, 6600. (c) Martin, S.F.; Pacofsky, G.J.; Gist, R.P.; Lee, W.C. *J. Am. Chem. Soc.* **1989**, *111*, 7634. (d) Shimshock, S.J.; Waltermire, R.E.; DeShong, P. *J. Am. Chem. Soc.* **1991**, *113*, 8791.

POLYMERIZATIONS IN SUPERCRITICAL CARBON DIOXIDE

Reported by Keith Hirsch October 20, 1994

INTRODUCTION

Growing concern over the effect of chemical waste on the environment has led to the development of processes that minimize the use of organic solvents.[1] For example, future legislative restrictions on the emission of solvents such as chlorofluorocarbons (CFCs) will necessitate the use of environmentally benign substitutes. One alternative to traditional solvents is supercritical carbon dioxide ($CO_{2(sc)}$), which has been used as an extraction medium and a chromatography eluent.[2] Despite research activity in these areas, the use of $CO_{2(sc)}$ as a reaction medium has been limited. Within the last ten years, however, environmental concerns have led to the use of $CO_{2(sc)}$ as an alternative to conventional organic solvents for the synthesis of some small molecules[3] as well as a number of polymers. CO_2 offers advantages in that it is inexpensive, inert under many reaction conditions, nontoxic, and easy to separate from reaction mixtures. In addition to the environmental benefits realized through the use of $CO_{2(sc)}$, the compressibility of supercritical fluids (SCFs) allows the optimization of reaction conditions to maximize yield, selectivity, and reaction rate as well as other reaction parameters. This review summarizes the properties of SCFs and the use of $CO_{2(sc)}$ as a reaction medium in different polymerization methods.

DEFINITION AND PROPERTIES OF SUPERCRITICAL FLUIDS

Figure 1 shows a traditional pressure vs. temperature phase diagram for CO_2. The diagram shows that increasing the pressure of gaseous CO_2 at constant temperature causes condensation to liquid CO_2. Condensation occurs at temperatures no higher than the critical temperature (T_c) in the phase diagram. The critical pressure (P_c) is the pressure required for liquefaction to occur at the critical temperature. Together, the critical temperature and pressure define the critical point of a substance. The liquid/vapor line on the phase diagram ends at the critical point where the two phases are indistinguishable. At temperatures and pressures above the critical point, the substance exists as a SCF with a density (typically 0.7 g/mL near the critical point)[4] approaching that of a liquid and a viscosity (10^{-4} $N{\cdot}s/m^2$)[4] nearing that of a gas. An important gas-like property of SCFs is their compressibility which allows the modification of physical properties such as density, solvent strength, and dielectric strength with only slight changes in pressure. Such modifications are most pronounced near the critical point. The "tunability" of the properties of SCFs can influence reaction rate constants[5] and equilibrium constants[6] as well as other reaction parameters. Therefore, the compressibility distinguishes SCFs as potentially useful reaction media. In contrast, the relative incompressibility of organic solvents and other liquids limits the tuning of the aforementioned reaction variables to the use of cosolvents or entirely different solvent systems.

Copyright © 1994 by Keith Hirsch

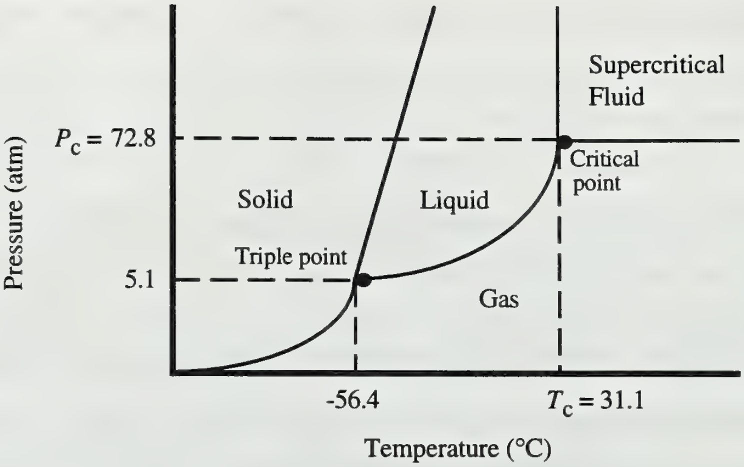


Figure 1. Phase diagram for CO_2 showing critical and triple points.

PRESSURE EFFECTS IN $CO_{2(sc)}$

One method used to probe solvent effects is solvatochromic characterization. In such studies, the change in the maximum absorption wavelength, λ_{max}, of a dye is monitored with changes in pressure of the SCF phase. In 1987, Kim and Johnston published solvatochromic data for phenol blue in $CO_{2(sc)}$ which demonstrated that increases in pressure led to stabilization of the excited state.[7] The two resonance structures for phenol blue are shown in Figure 2 and results of this study are summarized in Table I. The polarized resonance form makes a greater contribution to the resonance hybrid in the excited state than in the ground state.

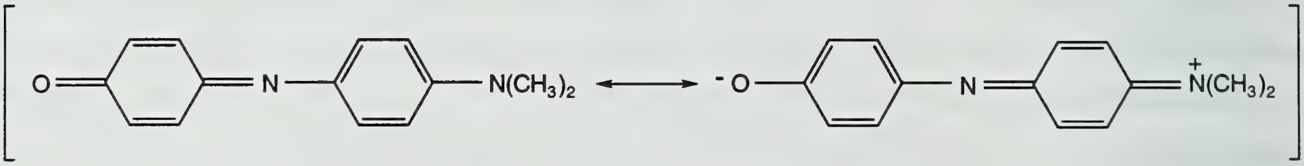


Figure 2. Resonance forms of phenol blue.

Since the transition energy is inversely proportional to λ_{max}, these data indicate that an increase in pressure results in a decrease in the transition energy. Thus, as the pressure increases, the excited state is stabilized to a greater extent. This observation was rationalized in terms of a clustering effect which creates a local

Table I. Solvatochromic Data for Phenol Blue in $CO_{2(sc)}$ at 35°C

Pressure (atm)	λ_{max} (nm)
74.0	518.7
83.8	526.9
197.3	533.4
295.9	535.8

density of CO_2 about phenol blue that is greater than the bulk density at a given pressure.[7,8] As the pressure increases, the clustering effect becomes more pronounced leading to greater stabilization of the excited state which is reflected in a shift to longer wavelength. Pressure variations at subcritical temperatures result in smaller increases in λ_{max} over a similar pressure range owing to the relative incompressibility of liquid CO_2 as compared to $CO_{2(sc)}$.

EARLY POLYMERIZATIONS AT SUBCRITICAL AND SUPERCRITICAL CONDITIONS

In 1968, Hagiwara and co-workers reported the first example of the free radical polymerization of ethylene in CO_2.[9] Their work described the formation of polyethylene initiated either by γ-radiation or 2,2'-azobisisobutyronitrile (AIBN) at 387.0 atm and temperatures ranging from 20-45°C. IR spectra obtained of the materials formed in CO_2 were identical to those obtained for the bulk polymerization indicating no CO_2 incorporation into the polymer backbone as evidenced by the absence of a carbonyl stretch at 1750-1700 cm^{-1}. The materials made in the presence of CO_2 were of lower molecular weight (204,000 g/mol with γ-radiation and 198,000 g/mol with AIBN) than the material obtained by the bulk process (360,000 g/mol). This observation was believed to result from a decreased rate of propagation in CO_2.

Kumar, et al. reported in 1987[10] that the precipitation polymerization of styrene in supercritical ethane affords low molecular weight polystyrene with a narrow molecular weight distribution.[11] During a precipitation polymerization, the reaction medium is initially homogeneous, but turns heterogeneous as the insoluble polymer forms. AIBN was used as an initiator at pressures ranging from 118.4-246.7 atm and temperatures between 60-70°C to produce polystyrene with an average molecular weight of 1000 g/mol and a polydispersity index of 1.2. This low polydispersity was obtained by altering the precipitation threshold of the polymer with changes in pressure and monomer concentration. That is, the degree to which polystyrene of different chain lengths partitions between the ethane and precipitate-phases could be tuned in order to minimize the molecular weight distribution. These studies demonstrated that the polydispersity increased with increasing pressure and monomer concentration. The rationale invoked to explain these observations was one of the SCF approaching liquid-like behavior as the compressibility of the SCF-phase decreases at high monomer concentrations and at pressures far removed from the critical point.

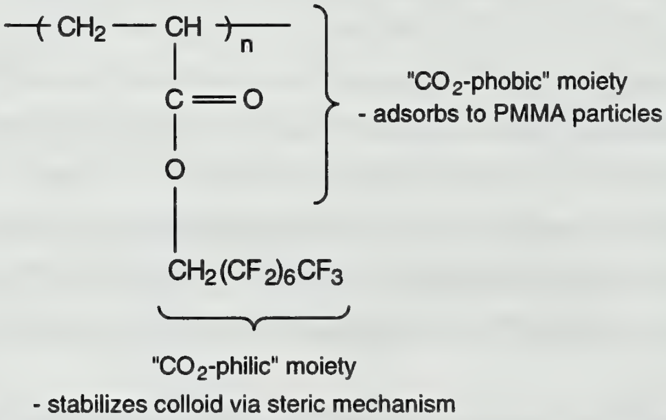
Further work in the formation of polystyrene under supercritical conditions was continued by Kiran and co-workers in 1990.[12] Polystyrene was prepared in supercritical *n*-butane at temperatures of 160-180°C and pressures ranging from 59.2-345.4 atm using *t*-butyl peroxide as an initiator. Weight-average molecular weights[11] varied from 9,500-18,200 g/mol and polydispersities ranged from 1.6-2.4. The highest yield of polystyrene obtained was only 20%. It was observed that for reactions performed at 160°C for 5 h, an increase in pressure led to increases in molecular weight, polydispersity, and yield. The increase in molecular weight at higher pressures was rationalized by comparison of the change in the volume of activation for propagation (ΔV^*_{prop}) to that for termination (ΔV^*_{term}). Since ΔV^*_{prop} is usually negative for free radical polymerizations and ΔV^*_{term} is positive, an increase in pressure is expected to favor propagation and suppress termination. As a result, the rate of polymerization increases at higher pressures as does the molecular weight.

THE USE OF $CO_{2(sc)}$ AS AN INERT POLYMERIZATION MEDIUM

Homogeneous, dispersion, inverse emulsion, and precipitation polymerizations in $CO_{2(sc)}$ have recently been demonstrated.[13-16] The ability to utilize both homogeneous and heterogeneous methods in $CO_{2(sc)}$ allows the preparation of a variety of commercially important polymers.

Homogeneous Polymerization of 1,1-Dihydroperfluorooctylacrylate (FOA)

Although most hydrocarbon polymers are insoluble in $CO_{2(sc)}$, many fluorinated polymers and other highly fluorinated compounds are soluble.[13,17] This is important from an environmental point of view in that fluorinated polymers are typically soluble only in CFCs.[18] In 1992, DeSimone, et al. reported the first homogeneous polymerization in an inert SCF.[13] The formation of poly(FOA) (see Figure 3) with a molecular weight of 270,000 g/mol was performed in $CO_{2(sc)}$ at 59.4°C and 204 atm for 48 h using AIBN as a free radical initiator. Isolation of the polymer, obtained in 65% yield, was facilitated by slowly venting the system allowing gaseous CO_2 to escape. Comparison of ^1H NMR and IR spectra of the polymer with those of poly(FOA) prepared in 1,1,2-trifluorotrichloroethane showed identical structure indicating that $CO_{2(sc)}$ was inert under these conditions. Recently, poly(FOA) has shown promise as a stabilizer for colloids obtained through dispersion polymerizations.[14] This application of poly(FOA) is discussed in the next section.



"CO2-phobic" moiety
- adsorbs to PMMA particles
"CO2-philic" moiety
- stabilizes colloid via steric mechanism

Figure 3. Structure of poly(FOA) indicating features which render it a colloidal stabilizer.

Dispersion Polymerization of Methyl Methacrylate

The free radical dispersion polymerization of methyl methacrylate in $CO_{2(sc)}$ to form high molecular weight poly(methylmethacrylate) (PMMA) was reported recently by DeSimone and co-workers.[14] In this process, the reaction medium is initially homogeneous as both the monomer and initiator are soluble, but turns heterogeneous owing to the insolubility of the PMMA formed.[11] Dispersion polymerizations are conducted in the presence of a stabilizer that, by the nature of its structure, is able to partition between the continuous phase ($CO_{2(sc)}$) and precipitate phase preventing particle aggregation. This system results in the formation of a fine colloidal dispersion containing micron-sized polymer particles. Poly(FOA) was used as the stabilizer through the adsorption of the "CO_2-phobic" segment (see Figure 3) to the dispersed PMMA particles and the projection of the "CO_2-philic" segment into the CO_2-phase. This method is referred to as a steric mechanism in that the long-chain "CO_2-philic" moieties prevent flocculation via repulsions during collisions.[14]

PMMA was prepared at 65°C and 201 atm for 4 h using AIBN as the initiator at various stabilizer concentrations. Polymerizations were conducted using poly(FOA) stabilizers of two different molecular weights - one of low molecular weight (LMW) (number-average molecular weight (M_n)[11] of 1.1×10^4 g/mol) and one of high molecular weight (HMW) ($M_n = 2.0 \times 10^5$ g/mol). Results of these reactions are shown in Table II.[14]

Table II. Results of Dispersion Polymerization of Methyl Methacrylate in $CO_{2(sc)}$ at Various Stabilizer Concentrations and Molecular Weights

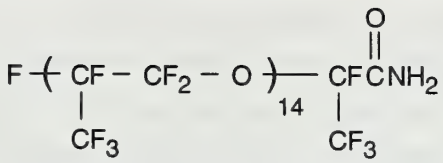
Stabilizer (w/v %)	Yield (%)	M_n (10^3 g/mol)	MWD*	Particle size (μm)
0%	39	149	2.8	-
2% LMW	85	308	2.3	1.2(±0.3)
4% LMW	92	220	2.6	1.3(±0.4)
2% HMW	92	315	2.1	2.7(±0.1)
4% HMW	95	321	2.2	2.5(±0.2)

* MWD refers to molecular weight distribution

Reactions conducted without added stabilizer proceeded as precipitation polymerizations resulting in low yields and low molecular weights. These results illustrate the formation of a stable colloid through the use of poly(FOA) as evidenced by the small particle sizes obtained which are similar to those achieved in traditional dispersion polymerizations.[19]

Inverse Emulsion Polymerization of Acrylamide

Recently, Adamsky and Beckman reported the first inverse emulsion polymerization of a water soluble monomer in $CO_{2(sc)}$ in the preparation of high molecular weight poly(acrylamide).[15] In this method, an aqueous solution of a hydrophilic monomer (acrylamide) forms an emulsion in a nonpolar solvent (CO_2) containing dissolved initiator. In this technique, a surfactant is added to stabilize the emulsion in a manner similar to that used in dispersion polymerization.[11] Amide functional poly(hexafluoropropylene oxide), shown in Figure 4, was used as a surfactant. The amide moiety acts as a polar head group (hydrophilic) and the polymeric moiety serves as a "CO_2-philic" (hydrophobic) tail.[15] Such an amphiphilic structure enables micelle formation which serves to stabilize the emulsion.



$$F\!\left(\underset{\underset{CF_3}{|}}{CF}-CF_2-O\right)_{14}\!-\underset{\underset{CF_3}{|}}{CF}\overset{\overset{O}{\|}}{C}NH_2$$

Figure 4. Structure of surfactant used for emulsion stabilization in formation of poly(acrylamide).

The inverse emulsion polymerization of acrylamide was carried out at 60°C and 340.2 atm for 1 h with AIBN as the initiator at various surfactant concentrations (see Table III). Similar to the formation of PMMA, polymerization in the absence of surfactant proceeded as a precipitation polymerization and resulted in a lower yield and lower molecular weight than the corresponding reaction utilizing a 1% (by mass) surfactant concentration.

Table III. Results of Inverse Emulsion Polymerization of Acrylamide in $CO_{2(sc)}$ at Various Surfactant Concentrations

Mass % Surfactant	$M_v \times 10^{-6}$ (g/mol)	Yield (%)
0%	6.61	91
1%	7.09	99.8
2%	4.92	99.8

The lower molecular weight polymer obtained using 2% surfactant was rationalized as possibly resulting from chain-transfer to the surfactant.[11,15] Molecular weights reported in this study are comparable to those obtained in hydrocarbon media.[20]

Precipitation Polymerization of Acrylic Acid

DeSimone and co-workers recently reported the formation of high molecular weight poly(acrylic acid) via precipitation polymerization in $CO_{2(sc)}$ using AIBN as a free radical initiator.[16] Reactions were performed at 55°C for 4 h with pressures varying from 108.5 to 335.4 atm (see Table IV). Increasing pressure had little effect on molecular weight or molecular weight distribution in these reactions. Also, the material obtained from a benzene control reaction was of lower molecular weight and lower molecular weight distribution. No explanation for this observed solvent effect was reported. Reactions were also conducted in the presence of varying concentrations of ethanethiol, a chain transfer agent, in order to obtain low molecular weight poly(acrylic acid) (see Table V).

Table IV. Results of Precipitation Polymerization of Acrylic Acid (AA) in $CO_{2(sc)}$ as Compared to Control Reaction in Benzene

Pressure (atm)	Mole Fraction AA/CO_2	$M_n \times 10^{-3}$ (g/mol)	MWD
108.5	0.118	149	3.9
202.2	0.083	144	3.8
266.3	0.075	146	3.9
335.4	0.071	146	3.8
Benzene control	-	135	2.8

It is evident that addition of a chain transfer agent enables the formation of low molecular weight poly(acrylic acid) with a narrow molecular weight distribution (0.169 *M* EtSH, e.g.). Tables IV and V illustrate the wide molecular weight range of acrylic acid polymers that can be prepared in $CO_{2(sc)}$.

Table V. Results of Precipitation Polymerization of Acrylic Acid in $CO_{2(sc)}$ in the Presence of a Chain Transfer Agent

[EtSH] *M*)	[AA] (*M*)	$M_n \times 10^{-3}$ (g/mol)	MWD
0.0012	1.34	153	2.88
0.0060	1.34	68.1	2.23
0.063	1.34	6.5	1.71
0.169	1.34	2.9	1.26

CONCLUSION AND FUTURE WORK

Supercritical fluids (SCFs) as reaction media offer the ability to influence or alter reaction parameters such as rate constants, equilibrium constants, and selectivities. This ability to tune SCFs is attributable to their compressible nature and is most evident through slight variations of pressure near the critical point where large changes in density are observed. Despite the advantages of using SCFs as solvents for polymerizations, their use has been limited. One reason for the lack of activity in this area is the large capital costs incurred in implementing high-pressure apparatus.[3e] However, increasing political pressure placed on the chemical industry to reduce solvent waste has led to the development of various polymerization processes utilizing $CO_{2(sc)}$ as an inert reaction medium. From an environmental point of view, it is necessary that CO_2 be recycled due to its implication in the greenhouse effect. Interestingly, this aspect of the use of $CO_{2(sc)}$ has not been adequately addressed in the literature.

The polymerizations performed in $CO_{2(sc)}$ through a variety of methods are characterized by high molecular weights and yields. While these preliminary results are encouraging, only a limited number of materials have been prepared. Thus, it is imperative that more research be conducted in order to determine the viability of $CO_{2(sc)}$ as a polymerization medium capable of replacing conventional liquid solvents for which the technology is well developed on the industrial scale. Toward this end, it is anticipated that new polymers with practical applications will be prepared in this medium along with continuing efforts to facilitate the preparation of known polymers.

REFERENCES

1. Illman, D.L. *Chem. Eng. News* **1994**, *72*(36), 22.
2. McHugh, M.A.; Krukonis, V.J. *Supercritical Fluid Extraction*; Butterworths: Stoneham, MA, 1986.
3. (a) Sigman, M.E.; Barbas, J.T.; Leffler, J.E. *J. Org. Chem.* **1987**, *52*, 1754. (b) Paulaitis, M.E.; Alexander, G.C. *Pure Appl. Chem.* **1987**, *59*, 61. (c) Randolph, T.W.; Clark, D.S.; Blanch, H.W.; Prausnitz, J.M. *Science* **1988**, *239*, 387. (d) Jessop, P.G.; Ikariya, T.; Noyori, R. *Nature*, **1994**, *368*, 231. (e) Kaupp, G. *Angew. Chem. Int. Ed. Engl.* **1994**, *33,* 1452.
4. Scholsky, K.M. *J. Supercrit. Fluids* **1993**, *6*, 103.
5. Johnston, K.P.; Haynes, C. *AIChE J.* **1987**, *33*, 2017.
6. Peck, D.G.; Mehta, A.J.; Johnston, K.P. *J. Phys. Chem.* **1989**, *93*, 4297.
7. Kim, S.; Johnston, K.P. *AIChE J.* **1987**, *33*, 1603.
8. Lemert, R.M.; DeSimone, J.M. *J. Supercrit. Fluids* **1991**, *4*, 186.
9. Hagiwara, M.; Mitsui, H.; Machi, S.; Kagiya, T. *J. Polym. Sci., Part A* **1968**, *6*, 603.
10. Kumar, S.K.; Suter, U.W. *Polym. Prep. (Am. Chem. Soc. Div. Polym. Chem.)* **1987**, *28(2)*, 286.
11. Odian, G. *Principles of Polymerizations*, 3rd ed.; Wiley-Interscience: New York, 1991.
12. Kiran, E.; Saraf, V.P. *J. Supercrit. Fluids* **1990**, *3*, 198.
13. (a) DeSimone, J.M.; Guan, Z.; Elsbernd, C.S. *Science* **1992**, *257*, 945. (b) Guan, Z.; Elsbernd, C.S.; DeSimone, J.M. *Polym. Prep. (Am. Chem. Soc. Div. Polym. Chem.)* **1992**, *33(2)*, 329.
14. (a) DeSimone, J.M.; Maury, E.E.; Menceloglu, Y.Z.; McClain, J.B.; Romack, T.J.; Combes, J.R. *Science* **1994**, *265*, 356. (b) Maury, E.E.; DeSimone, J.M. *Polym. Prep. (Am. Chem. Soc. Div. Polym. Chem.)* **1994**, *35(2)*, 868.
15. Adamsky, F.A.; Beckman, E.J. *Macromolecules* **1994**, *27*, 312.
16. Romack, T.J.; Maury, E.E.; DeSimone, J.M. *Polym. Prep. (Am. Chem. Soc. Div. Polym. Chem.)* **1994**, *35(2)*, 741.
17. (a) Consani, K.A.; Smith, R.D *J. Supercrit. Fluids* **1990**, *3*, 51. (b) Hoefling, T.; Stoefsky, D.; Reid, M.; Beckman, E.; Enick, R.M. *J. Supercrit. Fluids* **1992**, *5*, 237.
18. Wall, L. *Fluoropolymers, High Polymers*; Wiley-Interscience: New York, 1972; Vol. 25.
19. Tseng, C.M.; Lu, Y.Y.; El-Aasser, M.S.; Vanderhoff, J.W. *J. Polym. Sci., Part A* **1986**, *24*, 2995.
20. Hunkeler, D.; Hamielac, A.E.; Baade, W. *Polymer* **1989**, *30*, 127.

ANTISENSE OLIGONUCLEOTIDE ANALOGS AS POTENTIAL THERAPEUTIC AGENTS: SYNTHESIS AND PROPERTIES

Reported by Heather Sings October 27, 1994

INTRODUCTION

The treatment of disease by modulation of biological processes can be undertaken at the level of the gene, the mRNA, or the protein.[1] Since the 1970's, proteins associated with disease pathology have been a major focus of drug discovery effort. This methodology, however, suffers from several complications because the structure and mode of action of protein targets (enzymes, receptors, and ion channels) are often complicated and not well understood. Drugs which specifically target DNA have disadvantages as well. For instance, therapeutic agents such as adriamycin, bleomycin, or cisplatin interact with DNA base pairs in a non-sequence specific manner, causing many unwanted side effects due to the inability of these drugs to distinguish between normal and diseased cells.[2] More recently, a new strategy for controlling disease states has emerged which uses "antisense oligonucleotides" to modulate gene expression through direct interaction with mRNA. This review will focus on the synthesis and biological properties of antisense oligonucleotide analogs, as well as the mechanisms by which they control gene expression.

BACKGROUND

Definition

The term "antisense oligonucleotide" is derived from DNA replication. During replication, the strand that is acting as the template is called the "sense strand" while its complement is the "antisense strand". In the context of this review, an antisense oligonucleotide refers to a 15-20 nucleotide sequence of oligodeoxyribonucleotides (ODNs), which lacks coding capacity and bind to the complementary sense strand of their target mRNA. The antisense concept suggests that inhibition of gene expression may result from the Watson-Crick pair binding of these antisense ODNs to single-stranded RNA such that the normal, essential function of the RNA is disrupted (Figure 1).[3] If the target protein is a requirement for cell growth, or for intracellular viral replication, then these processes would be inhibited by the antisense agent.

Mechanism and requirements for gene inhibition

Although the precise mechanism for disruption of gene expression after sequence-specific binding of the synthetic antisense ODN to a target strand is not known, two types of termination events may be possible. The first denotes antisense ODN binding to the region of mRNA where

Copyright © 1994 by Heather Sings

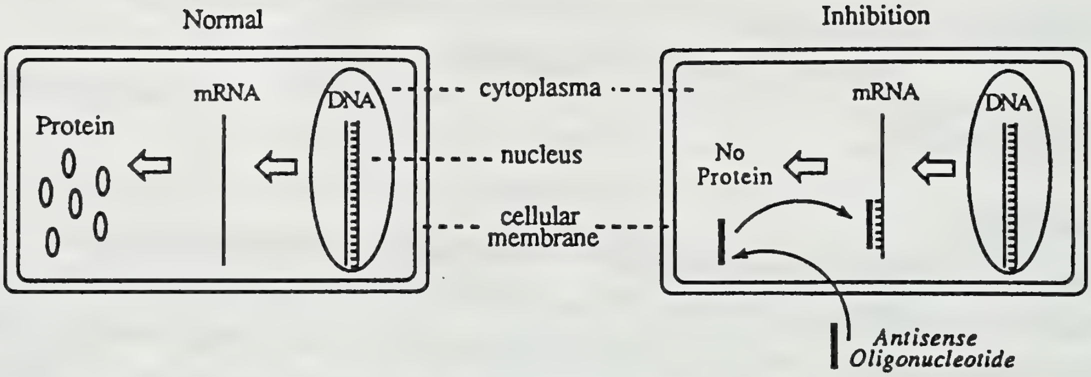


Figure 1. Principle of action of antisense oligonucleotides.[2]

protein translation begins. Antisense ODN binding to this area may inhibit translation by preventing the ribosomes and important initiation factors from interacting with the mRNA. The second type of termination event may occur through the action of ribonuclease H (RNaseH), a ubiquitous enzyme found in organisms as diverse as viruses to human cells. Its function in DNA replication is to hydrolyze the RNA phosphodiester bond, producing RNA fragments which are used as primers for DNA polymerase.[4] The action of RNaseH can be used in antisense therapy to cleave the mRNA of a ODN-mRNA duplex after the antisense strand has been bound to its mRNA target. Once cleaved, the mRNA is no longer competent for translation and is rapidly degraded. Inhibition of gene expression through the use of RNaseH hydrolysis may have an advantage because each segment of mRNA is permanently inactivated upon cleavage and each ODN can potentially inhibit multiple copies of target mRNA.[5]

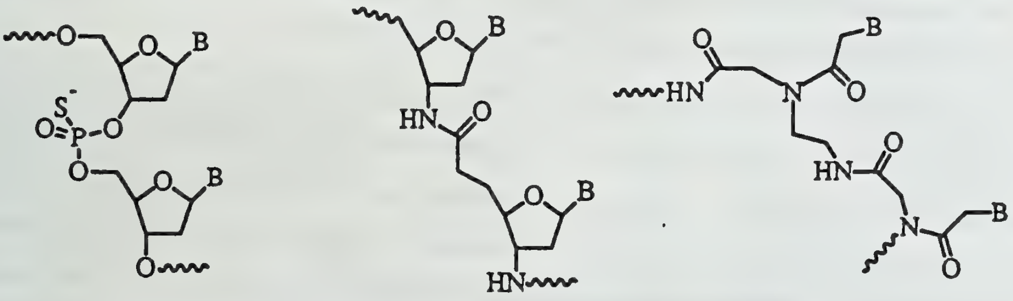
For an antisense ODN to inhibit gene expression effectively, it must fulfill the following criteria: 1) have the ability to cross the cell membrane and reach its target sequence within the cell; 2) have stability toward extra and intracellular nucleases; and 3) bind to its target sequence with appropriate kinetics, specificity, and affinity. Satisfaction of these requirements, necessitates chemical modification of the phosphodiester backbone, sugar, or heterocyclic base moieties.

Duplex stability and geometry

Chemical modifications of an ODN can have a dramatic effect on its binding with a target mRNA. Therefore, most modifications are directed toward the phosphate backbone which minimizes interference with Watson-Crick base pairing. One widely used method to evaluate affinity between two oligonucleotides is spectrophotometric analysis, where absorbance versus temperature is measured for a stoichiometric mixture of complementary ODNs. This technique, allows for the melting temperature (T_m) - the temperature at which 50% of the double strand has dissociated into its two single strands - to be measured. With the assumption of a two-state transition, thermodynamic parameters ($\Delta H°$, $\Delta S°$, $\Delta G°$) for duplex formation are also obtained.[6]

Molecular modeling can then be applied to gain insight into the shape and orientation of the helix formed by the antisense ODN and its target. In general, RNA-RNA and RNA-DNA duplexes exist in the A-form while DNA-DNA duplexes exist in the B-form. The principle difference between A and B helices is the sugar orientation which is C_3'-*endo* for A as opposed to C_2'-*endo* for B.[7] This difference in sugar orientation leads to differences in the overall shape of A and B form helices. Since antisense ODNs form DNA-RNA duplexes, backbone modifications which stabilize the A geometry are preferred.

Modifications of the phosphodiester backbone also increase nuclease stability, a major objective in the development antisense ODNs. Alterations of the sugar and base moieties as well as the attachment of chemically reactive groups and intercalating agents have also been explored and are reviewed elsewhere.[2] The remainder of this review will focus on the synthesis and properties of three backbone modified ODNs which show promise as antisense agents: phosphorothioates, amides, and peptide nucleic acids (Figure 2). Other interesting, but less well-characterized linkages derived from phosphodiester backbones include phosphoramidites, alkyl phosphotriesters, and boranophosphates.[2] Non-phosphorous backbone modifications include carbonates, carbamates, sulfur(II, IV, and VI), formacetal, oximes, and imines.[8]



Phosphorothioate ***Amide*** ***Peptide nucleic acid***

Figure 2. Antisense oligonucleotides with phosphorothioate, amide, and polyamide linkages. B equals one of the following bases: Adenine(A), Guanine(G), Cytosine(C), or Thymine(T).

ANTISENSE OLIGONUCLEOTIDES WITH PHOSPHOROTHIOATE LINKAGES

Phosphorothioates, one of the earliest analogs of naturally occurring phosphodiesters, have a phosphoryl oxygen replaced by sulfur. These antisense analogs show enhanced nuclease stability and cell permeation with only a slight decrease in duplex stability ($\Delta\Delta G^\circ_{37} = 0.3$ kcal/mol per modification) as compared to an unmodified ODN.[9] Currently there are two phosphorothioate analogs undergoing Phase II clinical trials: ISIS 2922 for human cytomegalovirus (CMV) and ISIS 2105 human papillomavirus (HPV).[10]

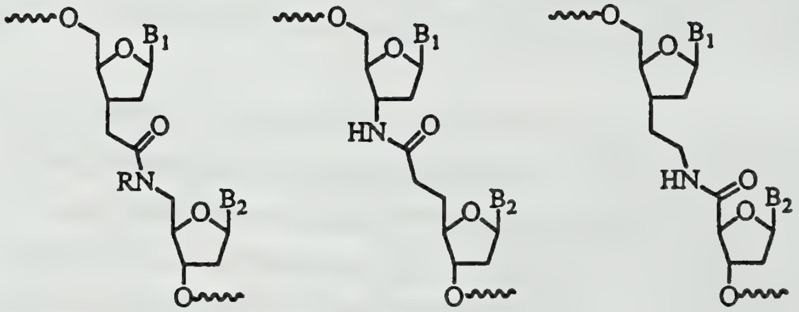
Phosphorothioate ODNs are synthesized using the solid-phase deoxynucleoside phosphoramidite approach.[11,12] It entails linking the first deoxyribonucleoside through its 3'-

hydroxyl to an inert, silica-based soluble support, which also acts as a 3'-blocking device. The 5'-hydroxyl group is subsequently deprotected and condensed with excess nucleoside 3'-cyanoethyl *N,N*-diisopropylphosphoramidite using tetrazole catalysis. The sulfur functionality is then introduced using 3*H*-1,2-benzodithiol-3-one 1,1-dioxide as a sulfur transfer reagent.[13] Any 5'- hydroxyl groups that failed to condense are capped as acetate esters. The cycle is repeated until desired chain length is reached with yields for each coupling and oxidation step ≥ 99%. The oligonucleotide is then deprotected and removed from the support using aqueous ammonia.

The replacement of oxygen by sulfur creates a stereogenic phosphorous center. Unfortunately, there is currently no method for the asymmetric synthesis of these backbone analogs. Thus, phosphorothioate analogs are made and evaluated as diastereomeric mixtures, which may account for the observed decrease in duplex stability. Molecular modeling studies on phosphorothioate-DNA duplexes have shown that phosphorothioate ODNs containing the *S* configuration at phosphorous have the same energy as phosphodiester backbones while the *R* configuration is greatly destabilized.[14] This is presumably attrituble to steric interference by the sulfur atom of the *R* isomer which is oriented into the major groove of the helix. However, the problem associated with diastereomeric mixtures is offset by the increased nuclease stability of phosphorothioate analogs which is caused by reduced dissociation rate (10-1000 fold compared to phosphodiester linkages) of the enzyme-thio-nucleotide complex.[15]

ANTISENSE OLIGONUCLEOTIDES WITH AMIDE LINKAGES

Replacing the phosphodiester linkage by an amide function represents another alternative which is more resistant to degradation than the phosphodiester linkage and is compatible with the conditions required for solid-phase synthesis. The amide linkages which have been synthesized and evaluated are depicted in Figure 3. Molecular modeling and binding affinity studies suggest that the orientation of the amide linkage has a very pronounced effect on duplex structure and stability.[16]



1 2 3

Figure 3. Amide linkages which have been synthesized and evaluated. R = H, Me, *i*-Pr.

The melting temperatures of the oligonucleotides containing amide **1** linkages (R=H) and their RNA or DNA counterparts are shown in Table 1. Incorporation of single or multiple thymine-amide-thymine or thymine-amide-cytosine dimers leads to an increase in thermal stability when complexed to RNA but a decrease in stability results from complexation to DNA. These binding data are quite different from those obtained for amides 2 and 3.[17] The incorporation of 2 or 3 into the same oligonucleotides led to an average drop in the T_m of the duplexes with RNA by -2.9 °C per modification for 2 and by -1.6 °C per modification for 3. Conformational analysis using AMBER force fields was carried out for each of the three amide modifications on hybrid duplexes by enforcing various torsion angles and relaxing the structure to the closest energy minimum.[16] These modeling studies suggest that the amide **1** modification can adopt a geometry which allows for adequate base-pair interaction between the antisense ODN-RNA duplex. This geometry more closely resembles the A form of RNA rather than the B form of DNA, which also explains why the amide **1** modification displays a higher affinity for RNA than DNA.

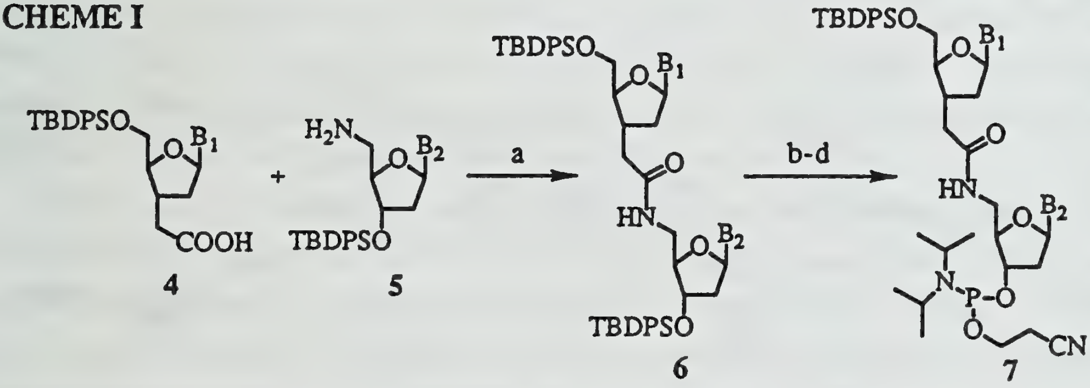
Table 1. Melting temperatures of duplexes of modified oligonucleotides containing amide-1 linkages and their RNA or DNA counterparts.

	Wild-type T_m (°C)	RNA complement ΔT_m	DNA complement ΔT_m
A. CTCGTACCTaTTCCGGTCC	63.3	+ 0.9	–
B. CTCGTACTaTTaTCCGGTCC	61.8	+ 0.5	–
C. TTTTTaTCTCTCTCTCT	51.6	+ 0.4	–
D. GCGTaTTaTTaTTaTTaTGCG	54.1	–	- 1.5
E. TTTTaTCTCTCTCT	42.3	–	- 1.7
F. TaTTaTTaCTaCTaCTaCTaCT	57.1	+ 0.6	--
TaTTaTTaCTaCTaCTaCTaCT	41.8		- 0.3

$\Delta T_m = [T_m(\text{modified oligonucleotide}) - T_m(\text{wildtype})]/(\text{number of modifications})$.

The synthesis of ODNs containing amide linkages is depicted in Scheme 1.[16, 18] Carboxylic acid 4 is activated with *O*-(1*H*-benzotriazolyl)-*N,N,N',N'*-tetramethyluronium

SCHEME I



(a) *N*-methylmorpholine, TBTU, *N*-hydroxybenzotriazole, MeCN, RT, 0.5 h, then X, *N*-methylmorpholine, MeCN, RT, 20 h. (b) $n\text{Bu}_4\text{NF}$, AcOH, THF ,RT, 20h.(c) DMTCl, pyridine, RT, 40h. (d) $(i\text{-Pr}_2\text{N})_2\text{POCH}_2\text{CH}_2\text{CN}$, $i\text{-Pr}_2\text{NH}_2^+$ tetrazole$^-$, CH_2Cl_2, RT, 40h.

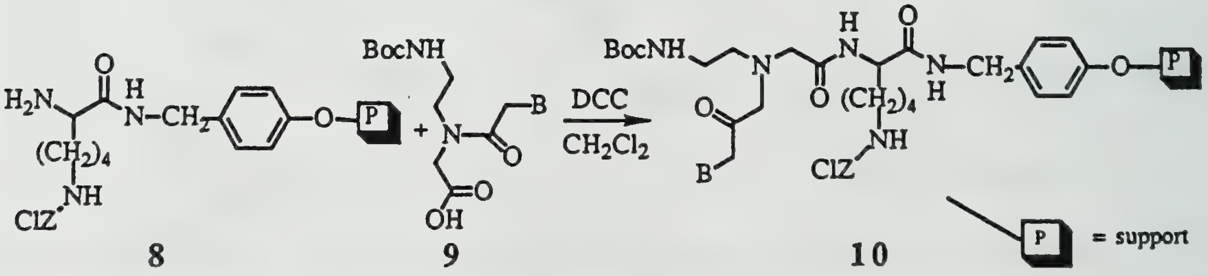
tetrafluoroborate (TBTU) and *N*-hydroxybenzotriazole and then coupled with amine 5. The removal of the silyl protecting groups with *n*-Bu_4NF in the presence of acetic acid is followed by the selective introduction of the dimethoxytrityl (DMT) group at the 5' position and the phosphoramidite function at the 3' position. The solid-phase synthesis of the ODNs and their purification can then be performed using standard phosphoramidite chemistry.

PEPTIDE NUCLEIC ACIDS (PNAs)

PNAs have attracted much attention since they were first introduced by Nielson *et al.* (Figure 2).[19, 20] They were designed using a computer model in which the backbone of one strand in a B-DNA duplex was detached and replaced with amino acid units. The optimal number of bonds between the nucleobases was found to be six, which corresponds to that in DNA, and the optimal number of bonds between the backbone and nucleobase to be two to three. The resulting molecule is composed of a backbone containing *N*-(2-aminoethyl)glycine units in which the nucleobase is attached to the glycine nitrogen via a methylene carbonyl linkage. PNAs possess several unique properties including: 1) binding of complementary DNA or RNA strands with significantly higher affinities than their oligonucleotide counterparts; 2) discrimination against mismatches as effectively as, or even better than, unmodified oligonucleotides; 3) resistance to nucleases; 4) preparation on large scale with relatively low cost.[21]

PNAs are prepared by the stepwise coupling of monomer 9 using Merrifield's solid-phase approach for peptide synthesis (Scheme II).[22] C-terminal amides are obtained by assembling the protected PNAs on a *p*-methoxybenzylamine resin initially loaded with Boc-L-Lys(ClZ).[23] The lysine included at the C-terminus diminishes PNA self-aggregation and allows for the determination of overall efficiency by amino acid analysis. The progress of the PNA synthesis is monitored throughout by quantitative ninhydrin analysis which shows that all couplings proceed with an efficiency of ≥ 99%.[24] Deprotection and release of free PNA from the resin is accomplished with anhydrous HF.

SCHEME II



PNA-RNA and PNA-DNA duplexes: structure and stability.

PNA's form duplexes of higher thermal stability than the corresponding DNA-DNA or DNA-RNA duplexes.[25] Thermodynamic parameters for hybridization were extracted from T_m measurements to determine ΔH°, ΔS° and ΔG° for formation of the PNA-RNA, DNA-RNA, PNA-DNA and DNA-DNA duplexes (Table 2). Free energy differences indicate that PNA's display a higher affinity for DNA or RNA than their unmodified counterpart. The decrease in entropy is almost identical for the formation of DNA-DNA and PNA-PNA duplexes which implies that the single-stranded PNA has the same degree of organization as single-stranded DNA.

Table 2. Thermodynamic parameters for the formation of PNA-DNA, PNA-RNA, DNA-RNA and DNA-DNA duplexes with the sequence TCTACGTCACAACTA present in the PNA strand

	DNA:RNA	PNA:RNA	DNA:DNA	PNA:DNA
ΔH° (kcal mol $^{-1}$)*	-128.9	-128.5	-105.3	-106.6
ΔS° (EU)*	-372.8	-345.9	-296.2	-285.8
ΔG°_{37}(kcal mol $^{-1}$)*	-13.3	-21.2	-13.4	-18.0
T_m (°C, 8 μm)*	50.1	72.2	53.5	68.8

Measured in 100mM NaCl, 10 mM Na_2PO_4, 0.1mM EDTA, pH 7.0
* Obtained from linear plots of $1/T_m$ versus log(concentration)

Since the PNA backbone is achiral, there is *a priori* no reason why binding to complementary oligonucleotides should occur in only one (antiparallel) orientation as is the case for DNA or RNA (considered antiparallel orientation if the amino terminus of the PNA is facing the 3'-end of the oligonucleotide). Information about orientation was obtained from CD and NMR experiments (HMQC, NOESY,TOCSY).[26, 27] The spectra indicate that PNAs form 1:1 complexes with complementary RNA which are antiparallel, right-handed double helices with Watson-Crick base pairing similar to the A form of RNA duplexes. T_m measurements confirmed that PNAs form more stable duplexes with their RNA counterparts when bound in the antiparallel orientation (T_m antiparallel = 69.5°C, T_m parallel = 56.1°C).

Although very few results concerning the antisense action of PNA have been reported, *in vitro* translation experiments have shown that a truncated protein product corresponding to translational interference at the PNA binding site is produced with a 20-mer PNA.[28] What has yet to be established is cellular and *in vivo* activity of PNAs. These data will be the key to determining the real potential of PNAs, however for now PNAs appear to be promising candidates for antisense drugs due to their unique binding properties.

CONCLUSION

Antisense technology represents a novel approach for controlling the translation of a protein. Phosphorothioates, amides, and peptide nucleic acids provide evidence that specific features of ODNs can be altered to increase binding affinity and nuclease resistance. The encouraging finding from the work presented in this review is how tolerant the process for base pairing is toward chemical modification of the phosphodiester backbone. Antisense oligonucleotide analogs show great potential as therapeutic agents. However, even if they fail as drugs, they can find use as molecular biology tools to elucidate enzymatic mechanisms, or as DNA probes.

REFERENCES

1. Kiely, J. S. In *Annual Reports in Medicinal Chemistry* Acedemic Press, New York, 1994, *29*, 297.
2. Uhlmann, E.; Peyman, A. *Chemical Reviews* 1990, *90*, 578.
3. Cook, P. D. *Anti-Cancer Drug Design* 1991, *6*, 585.
4. Hostomska, Z.; Matthew, D. A. *Nucleases;* Cold Spring Harbor Laboratory Press, New York, 1993.
5. Crooke, S. T. *FASEB* 1993, *7*, 533.
6. Albergo, D. D.; Marky, L. A.; Breslauer, K. F.; Turner, D. H. *Biochemistry* 1981, *20*, 1409.
7. Blackburn, M. B.; Gait, M.J. *Nucleic Acids in Chemistry and Biology*; IRL Press, New York, 1990.
8. Vama, R. S. *SynLett* 1993, *621*, 1993.
9. Erickson, R. P. *Gene Regulation*; Raven Press, New York, *95*, 1993.
10. ISIS Pharmaceuticals, Carlesbad California. Annual Report 1994.
11. Matteucci, M. D.; Caruthers, J. *J. Am. Chem. Soc.* 1981, *103*, 3185.
12. Sinha, N. D.; Biernat, J.; Koster, H. *Nucleic Acids Research*, 1984, *12*, 4537.
13. Iyer, P. R.; Phillips, L. R.; Egan W.; Regan, J. B.; Beucage, S. *J. Org. Chem.* 1990, *55*, 4693
14. Jaroszewski, J. W.; Syi, J.; Maizel, J.; Cohen, J. *Anti-Cancer Drug Design* 1992, *7*, 253.
15. Eckstein, J. *Angew. Chem. Int. Ed. Engl.* 1975, *14*, 367.
16. De Maeker, A.; Waldner, A.; Lebreton, J.; Hoffmann, P.; Fritsch, V.; Wolf, R.; Freier,S. *Angew. Chem. Int. Ed.* 1994, 33, 226.
17. De Mesmaeker, A.; Lebreton, J.; Waldner, A.; Fritsch, V.; Wolf, R. *Biorg. Med. Chem. Lett.* 1994, 4, 873.
18. Lebreton, J.; Waldner, A.; Lesueur, C.; De Mesmaeker, A. *SynLett* 1994, 137.
19. Egholm, M.; Buchardt, O.; Nielsen, P.; Berg, R. *J. Am .Chem .Soc.* 1992, *114*, 1895.
20. Egholm, M.; Nielsen, P.; Buchardt, O.; Berg, R. *J. Am Chem Soc*, 1992, *114*, 9677.
21. Nielsen, P.; Egholm, M.; Buchardt, O. *Bioconjugate Chem*, 1994, *5*, 3.
22. Merrifield, R.,B. *J. Am .Chem .Soc.* 1963, *85*, 2149.
23. Pietta, P.G.; Marshall, G. R. *J Chem Soc. D* 1970, 650.
24. Sarin, V.,K.; Kent, S.; Tam, J.,P.; Merrifield, R. B. *Analytical Biochemistry* , 1981, *117*, 147.
25. Egholm, M.; Buchardt, O.; Christensen, L.; Behrens, C.; Freier, S.; Driver, D. A.; Berg, R. H.; Sin, S. K.; Norden, B.; Nielsen, P. E. *Nature*, 1993, *365*, 566.
26. Brown, S. C.; Thomson, S. A.; Veal, J. M.; Davis, D. G.; *Science*, 1994, *265*, 777.
27. Hanver, J. C.; Peffer, N. J.; Bisi, J. E.; Thomson, S. A.; Cadilla, R.; Josey, J.A.; Ricca, D. J.; Hassman, F.; Bonham, M. A.; Au, K. G.; Carter, S.G.; Bruckenstein, D.A.; Boyd, A. L.; Noble, S. A.; Babiss, L. E. *Science* 1992, *258*, 1481.

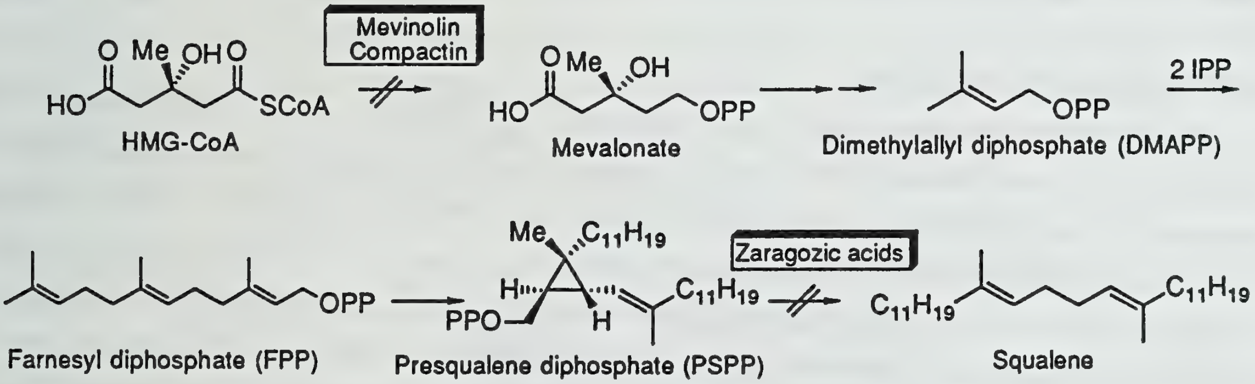
THE ZARAGOZIC ACIDS: A NEW CLASS OF POLYKETIDE DERIVED SQUALENE SYNTHASE INHIBITORS

Reported by Alexandra Katauskas November 3, 1994

INTRODUCTION

Coronary heart disease is the major cause of death and cardiovascular morbidity in the western world. A primary risk factor in the progression of the disease is hypercholesterolemia. Pharmacological reduction of serum cholesterol levels has been achieved by inhibiting cholesterol biosynthesis. Research efforts in this area have primarily focused on the inhibition of HMG-CoA reductase, a major regulatory enzyme on the cholesterol biosynthetic pathway. Inhibitors of this enzyme, such as compactin[1] and mevinolin,[2] are effective therapeutic agents. However, inhibition of HMG-CoA reductase occurs well before the formation of squalene in the biosynthetic pathway, and therefore, may adversely affect the formation of other biologically important compounds. Squalene synthase (SS) catalyzes the two-step conversion of farnesyl diphosphate (FPP) to squalene.[3] (Scheme I) As this is the first step after the branchpoint in the pathway to form other isoprenoids, it serves as a better target for specific inhibition of sterol synthesis. This review will discuss various aspects of a recently discovered class of squalene synthase inhibitors, the zaragozic acids (ZAs).

Scheme I


Mevinolin
Compactin
HMG-CoA
Mevalonate
Dimethylallyl diphosphate (DMAPP)
2 IPP
Zaragozic acids
Farnesyl diphosphate (FPP)
Presqualene diphosphate (PSPP)
Squalene

ISOLATION

During a screening campaign for SS inhibitors, the Bergstrom group at Merck[4] isolated three structurally related inhibitors, the zaragozic acids A(1), B(2), and C(3). Zaragozic acid A is produced by a sterile fungus ATCC 20986, while zaragozic acids B and C are produced by *Sporormiella intermedia* and *Leptodontium elatius,* respectively. Isolation of the ZAs was performed through a four-step

Copyright © 1994 by Alexandra Katauskas

procedure by taking advantage of their amphoteric nature. A group of compounds named the squalestatins, with squalestatin 1 identical to zaragozic acid A, was independently isolated from a strain of the fungus *Phoma*.[5,6] More recently, Hasumi et al. at Tokyo Noko University[7] have also isolated zaragozic acid A from a different microorganism, *Setosphaeria khartoumensis*. These compounds, whether designated the squalestatins or the zaragozic acids, incorporate the same highly functionalized bicyclic ketal core possessing three carboxylic acid groups, two hydroxy groups, a lipophilic side chain, and an α, β-unsaturated ester side chain.

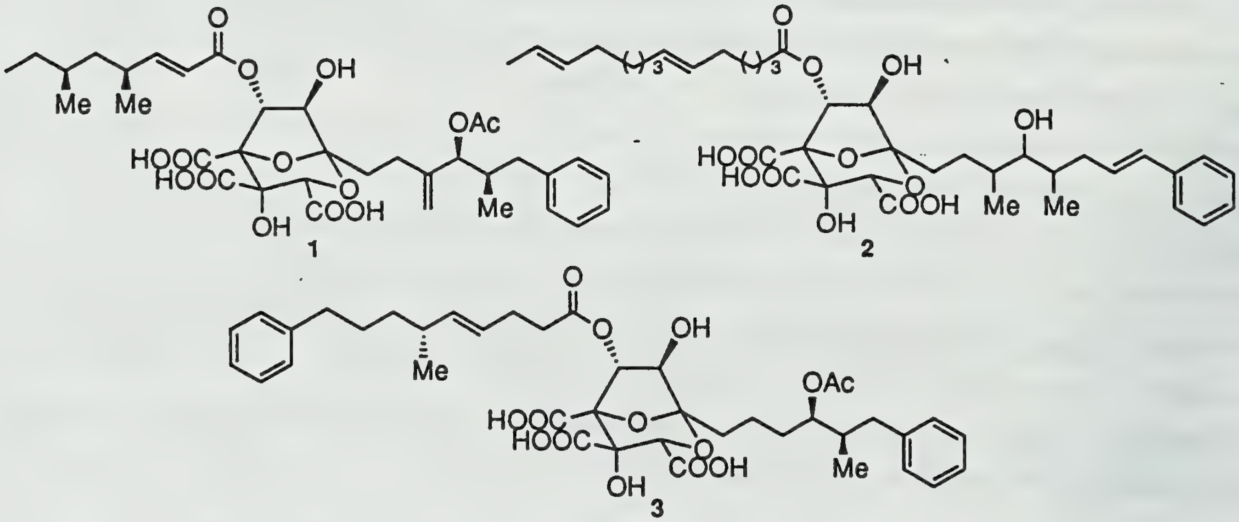


Figure 1. The Structures of Zaragozic Acids A, B, and C (**1**, **2**, and **3**).

STRUCTURE ELUCIDATION

The structures of the ZAs were determined by a combination of spectroscopic, X-ray crystallographic, and chemical methods.[8] Various functional groups were deduced from one dimensional ^1H and ^{13}C NMR spectra while derivatization identified the presence of three carboxylic acids and two hydroxyl groups. Two-dimensional NMR experiments revealed the structure of the two alkyl side chains. The sites of attachment of these two side chains were determined by a heteronuclear NOE difference experiment. Secondary isotope multiplet NMR was used to gain information about the bicyclic core while the full relative stereochemistry was established by a single-crystal X-ray diffraction study. The absolute configuration was determined by degradation to (2*S*,4*S*)-2,4-dimethylhexanoic acid. The structures of related compounds, **2** and **3** and various other minor metabolites,[9] were deduced in a similar manner by spectral comparisons with spectra of **1**.

BIOLOGICAL ACTIVITIES

The zaragozic acids exhibit nanomolar inhibitory activity against both mammalian (rat liver) and fungal (*Candida albicans*) SS.[10] More importantly, in marmosets, a species with a lipoprotein metabolism similar to that of man, the zaragozic acids lower serum cholesterol by up to 75%.[11] These compounds also display a broad spectrum of antifungal activity at concentrations as low as 0.25 µg/mL.[10] Furthermore, although less potent, this class of compounds has shown activity against farnesyl protein transferase and thus, holds promise as potential anticancer agents.[12]

MECHANISM OF ACTION

Although the mechanism of action is not fully understood, it has been postulated that the zaragozic acids inhibit squalene synthase by mimicking the binding of intermediate PSPP (Scheme I) to the enzyme. The two lipophilic side chains of the ZAs are thought to mimic the farnesyl-derived side chains of PSPP. More specifically, Procopiou and co-workers[13] proposed that the phenyl ring may provide additional binding to the enzyme in a manner analogous to the double bonds found in the side chain of PSPP. In addition, the tricarboxylic acid function may provide actions similar to those of the diphosphate moiety of the intermediate.

BIOSYNTHESIS

The particularly complex substitution pattern in this family of compounds stimulated an extensive study to determine their biosynthetic origin.[14] Researchers at Merck used a combination of radiolabelled precursors to obtain information about the derivation of these compounds. The results indicate the main carbon skeleton is derived from two acetate-generated polyketide chains. Interestingly, one of the chains appears to be derived from benzoic acid, itself derived from metabolism of phenylalanine. The remaining carbons arise from methionine and a four-carbon unit derived from succinate. A total of five of the oxygens, including the heterocyclic oxygens, are produced from atmospheric oxygen while the two oxygens at the ester carbonyls are derived from acetate. The proposed biosynthetic origin of **1** is summarized in Figure 2.

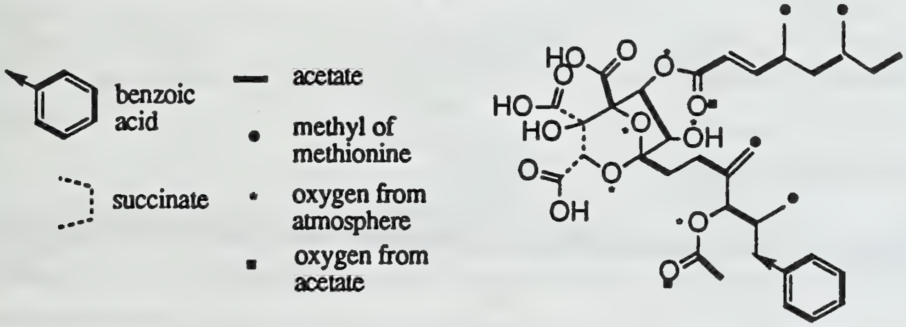
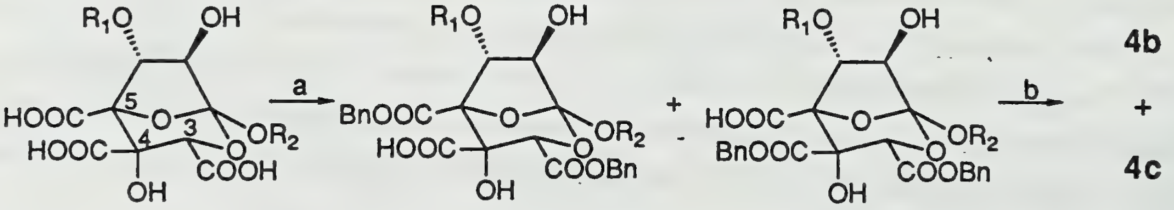


Figure 2. Proposed Biosynthetic Origin of Zaragozic acid A.

STRUCTURE-ACTIVITY RELATIONSHIPS

In an effort to gain insight into the mechanism involved in binding between the enzyme and the ZAs, several research groups have undertaken studies to determine the key structural features which are responsible for the biological activity.[14] A major objective of these researchers has been to obtain analogues which not only produce minimal side effects, but also possess maximum potency while being orally active.

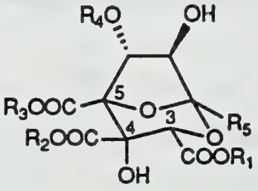
Selective modification of the carboxylic acid groups and the resulting effect on SS inhibitory activity has been reported by various groups.[15] Biftu et al.[15a] utilized a modification procedure in which the key step is selective debenzylation via transfer hydrogenolysis. A representative example is shown in eq. 1.



(1)

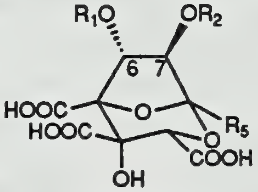
Reagents a) (i) BnOH, 3% HCl; (ii) DBU, BnCl; **b)** (i) MeOH, 3% HCl; (ii) *O*-methyl-*N*, *N'*-diisopropylisourea (R_1 and R_2= side chains of natural product **1**)

Compounds modified at positions C3 and C4 retain significant SS inhibitory activity with enhanced oral potency (**4a, 4b**). In contrast, esterification at the C5 position results in a loss of activity (**4c**). Diesters of the carboxylic acids at C3 and C4 enhance the *in vivo* activity over that of the parent natural product, although enzyme potencies are reduced. Chiang et al.[16] extended these studies by examining other diester derivatives. One particular diester combination (**4d**) was found to have a 16-fold increase in *in vivo* activity. Esterification at the C3 and C5 positions did not show the same enhanced activity (**4e**). Activities of the esterified derivatives are summarized in Table I.

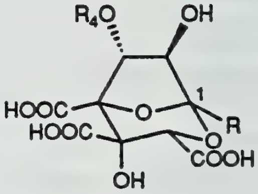


4 a-e

(R_4 and R_5 = side chains of natural product **1**)



5 a-e



6 a-b

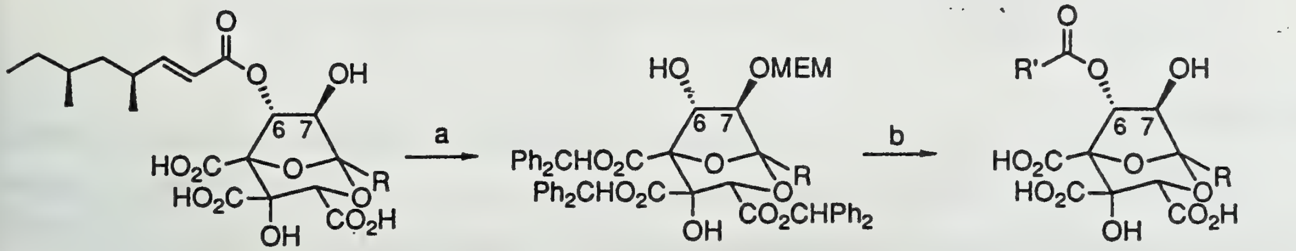
TABLE I. Activities of C3-C5 Carboxylate Ester Derivatives of Zaragozic Acid A (1 and 4a-4e).

compound number	R_1	R_2	R_3	RLSS IC_{50}(nM)	*in vivo* mouse mg/kg
1	H	H	H	12	100
4a	CH_3	H	H	7	24
4b	H	CH_3	H	4	40
4c	H	H	CH_3	NA[a]	NA
4d	$CH_2CH_2CH(CH_3)_2$	CH_2OCOCH_3	H	NA	6
4e	$CH_2CH_2CH(CH_3)_2$	H	CH_2OCOCH_3	NA	NA

[a] NA=not active at 13nM in rat liver squalene synthase assay (RLSS) or less than 50% inhibition at 40mg/kg in oral mouse assay

The nature of the C6 group has a critical impact on the tolerance towards modifications made elsewhere in the molecule. For example, modifications made to the carboxylic acids at C3 and C4, or the hydroxy group at C7 are well tolerated in the presence of a C6 ester functionality. However, upon removal of the C6 ester substituent, similar modifications result in a reduction of SS inhibitory activity.[17]

Giblin et al.[18] developed a protection/deprotection strategy that allows transformation of **1** into C6 acyl analogues (eq. 2). Simple modification of this protection strategy enables selective functionalization of the C7 hydroxyl group.



(2)

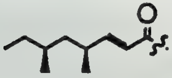
Reagents: a) (i) MEMCl, iPr_2EtN; (ii) Ph_2CN_2; (iii) MeNHOH.HCl, Et_3N; b) (i) R'COCl, DMAP; (ii) HCO_2H, H_2O (R= side chain of natural product)

The results indicate that derivatives containing small substituents at C7 retain nanomolar activity only in the presence of the lipophilic group at C6 (**5a**, **5b**). In contrast, large substituents at C7 are much less active (**5c**), regardless of the grouping at C6. Interestingly, the parent natural product possessing a hydroxyl group at C6 (**5d**)[5] and a number of derivatives containing different lipophilic fatty esters at C6 (**5e**)[18] retain potent activity. Activities of such derivatives are summarized in Table II.

TABLE II. *In vitro* Activities of C6 and C7 Modified ZAs (**1** and **5a-5e**).

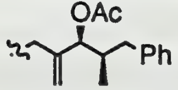
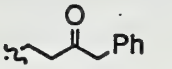
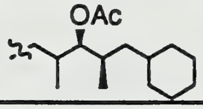
compound number	R_1	R_2	IC_{50} (nM) Rat liver SS	*C. albicans* SS
1	S*	H	12	5
5a	S*	OCO_2CH_3	15	15
5b	H	OCO_2CH_3	380	>200
5c	S*	$OCO(CH_2)_6CH_3$	>500	>200
5d	H	H	26	not tested
5e	$OCO(CH_2)_3CH_3$	H	6	26

S* =



Modifications made to the C1 side chain are not as easily tolerated. Any shortening of the carbon chain length (**6a**) or removal of the terminal phenyl ring (**6b**) results in a decrease in enzyme inhibition.[13, 19]

TABLE III. *In vitro* Activities of C1 Modified ZAs (**1** and **6a-6b**).

Compound number	R	IC_{50}(nM) Rat liver SS	 *C. albicans* SS
1		12	5
6a		>500	>300
6b		165	93

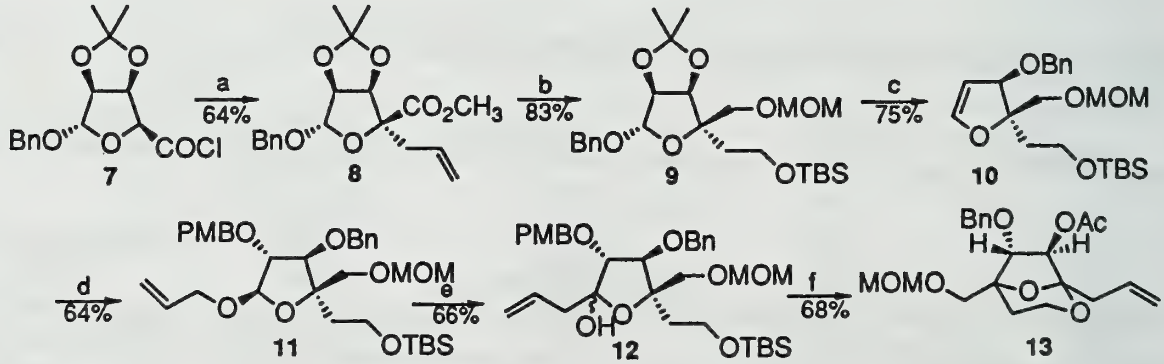
SYNTHESIS

The unique, highly oxygenated, bicyclic ketal of the zaragozic acids make this class a challenge to synthesize. To date, there has yet to be reported the total synthesis of the ZAs. However, several groups have pursued the task of synthesizing the novel 2, 8-dioxabicyclo[3.2.1]octane core,[20] while others have sought the asymmetric synthesis of the side chains.

Bicyclic Ketal Nucleus

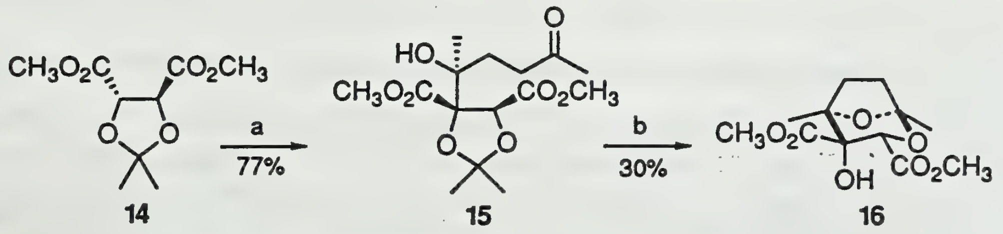
The McVinish group at the University of Melbourne[21] utilized a synthetic scheme starting from the known acid chloride **7**. (Scheme II) Conversion of **7** into a model core system proceeded via an Ireland-Claisen rearrangement to provide ester **8** and a stereoselective allylation of the furanoid glycal **10**.

Scheme II



Reagents: a) (i) Allyl alcohol, DMAP, CH_2Cl_2;(ii) LDA, TMSCl, HMPA/THF; CH_2N_2; b) (i) $LiAlH_4$, Et_2O; (ii) MOMCl, iPr_2NEt, CH_2Cl_2; (iii) OsO_4, NMO, THF/H_2O; (iv) $NaIO_4$; (v) $NaBH_4$, EtOH; (vi) TBSCl, imidazole, DMF; c) (i) Li, liq NH_3; (ii) HMPT, CCl_4, THF; (iii) Li, liq NH_3; (iv) BnBr, NaH, DMF/THF; d) (i) 2,2-dimethyldioxirane, acetone (ii) Allyl alcohol; (iii) PMBCl, NaH, DMF/THF; e) (i) $(Ph_3P)_3RhCl$, 1,4-diazabicyclo[2.2.2]octane, EtOH; (ii) $Hg(OAc)_2$, THF/H_2O; (iii) Allylmagnesium chloride, THF; (iv) Dess-Martin periodinane; f) (i) 50% HF/MeCN/H_2O; (ii) DDQ/CH_2Cl_2/H_2O; (iii) Ac_2O, pyridine

Aggarwal et al.[22] began the synthesis with a tartaric acid derivative (**14**) and acetonylacetone. A mixture of isomeric adducts was obtained upon addition of excess of tartrate anion, in the presence of 12-crown-4, to acetonylacetone. The mixture, when treated with acid, afforded a mixture of cyclized adducts, one of which possessed the bicyclic core of ZAs. Eq. 3 outlines the asymmetric synthesis showing only the product having the natural stereochemistry.

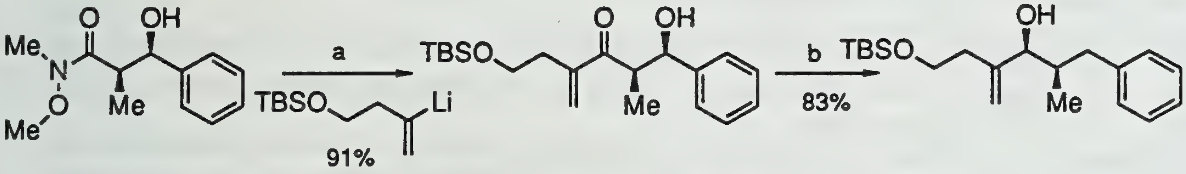


(3)

Reagents: a) LDA, THF, 12-crown-4, acetonylacetone; b) HCl, MeOH

C1 Side Chain

The asymmetric synthesis of the C1 side chain, performed by Evans et al.,[23] involved reaction of a known Weinreb amide with a TBS ether (eq. 4). Chelation-controlled reduction followed by formation of the acetonide and deoxygenation with Li/NH_3 produced the desired derivative.



(4)

Reagents: a) RLi, THF; b) (i) Et_2BOMe, $NaBH_4$; (ii) $(MeO)_2C(Me)_2$, PPTS, (iii) Li, NH_3, THF

SUMMARY

The ZAs , the first natural products to be potent inhibitors of SS, possess potential as therapeutic agents for the treatment of hypercholesterolemia. Although important information concerning structure-activity relationships has been obtained, future work must be performed to understand fully the mechanism responsible for biological activity. In addition, the highly functionalized bicyclic core of the ZAs pose challenges toward their total synthesis.

REFERENCES

(1) Abe, I.; Tomesch, J. C.; Wattanasin, S.; Prestwich, G. D. *Nat. Prod. Rep.* **1994**, *11*, 279.

(2) Alberts, A. W.; Chen, J.; Kuran, G.; Hunt, V.; Huff, J.; Hoffman, C.; Rothrock, J.; Lopez, M.; Joschua, H.; Harris, E.; Patchett, A.; Monaghan, R.; Currie, S.; Stapley, E.; Hensens, O.; Hirschfield, J.; Liesch, J.; Springer, J. *Proc. Natl. Acad. Sci. USA* **1980**, *77*, 3957.

(3) a) Poulter, C. D. *Acc. Chem. Res.* **1990**, *23*, 70. b) Poulter, C. D.; Robinson, G. W.; Jennings, S. M.; Zhang, D. *Arch. Biochem. Biophys.* **1993**, *304*, 133.

(4) Bergstrom, J. D.; Hensens, O. D.; Huang, L.; Liesch, J. M.; Onishi, J. C.; VanMiddlesmith, F. L.(1992) U. S. Patent 5,096,923.

(5) Dawson, M. J.; Farthing, J. E.; Marshall, P. S.; Middleton, R. F.; O'Neill, M. J.; Shuttleworth, A.; Stylli, C.; Tait, R. M.; Taylor, P. M.; Wildlman, H. G.; Buss, A. D.; Langley, D.; Hayes, M. V. *J. Antibiot.* **1992**, *45*, 639.

(6) Sidebottom, P. J.; Highcock, R. M.; Lane, S. J.; Procopiou, P. A.; Watson, J. S. *J. Antibiot.* **1992**, *45*, 648.

(7) Hasumi, K.; Tachikawa, K.; Sakai, K.; Murakawa, S.; Yoshikawa, N.; Kumazawa, S.; Endo, A. *J. Antibiot.* **1993**, *46*, 689.

(8) a) Wilson, K. E.; Burk, R. M.; Biftu, T.; Ball, R. G.; Hoogsteen, K. *J. Org. Chem.* **1992**, *57*, 7151. b) Hensens, O. D.; Dufresne, C.; Liesch, J. M.; Zink, D.; Reamer, R. A.; VanMiddlesworth, F. *Tetrahedron Lett.* **1993**, *34*, 399.

(9) Blows, W. M.; Foster, G., Lane, S. J.; Noble, D.; Piercey, J. E.; Sidebottom, P. J.; Webb, G. *J. Antibiot.* **1994**, *47*, 740.

(10) Baxter, A.; Fitzgerald, B. J.; Hutson, J. L.; McCarthy, A. D.; Motteram, J. M.; Ross, B. C.; Sapra, M. Snowden, M. A.; Watson, N. S.; Williams, R. J.; Wright, C. *J. Biol. Chem.* **1992**, *267*, 11705.

(11) a) Dufresne, C.; Wilson, K. E.; Singh, S. B.; Zink, D. L.; Bergstrom, J. D.; Rew, D.; Polishook, J. D.; Meinz, M.; Huang, L.; Silverman, K. C.; Lingham, R. B. *J. Nat. Prod.* **1993**, *56*, 1923. b) Gibbs, J. B.; Pampliano, D. L.; Mosser, S. D.; Rands, E.; Lingham, R. B.; Singh, S. B.; Scolnick, E. M.; Kohl, N. E.; Oliff, A. *J. Biol. Chem.* **1993**, *268*, 7617.

(12) Procopiou, P. A.; Bailey, E. J.; Hutson, J. L.; Kirk, B. E.; Sharratt, P. J.; Spooner, S. J.; Watson, N. S. *BioMed. Chem. Lett.* **1993**, *3*, 2527.

(13) a) Byrne, K. M., Arison, B. H.; Omstead, M. N.; Kaplan, L. *J. Org. Chem.* **1993**, *58*, 1019. b) Jones, C. A.; Sidebottom, P. J.; Cannell, R. J. P.; Noble, D.; Rudd, B. A. *J. Antibiot.* **1992**, *45*, 1492.

(14) a) Kuo, C. H.; Plevyak, S. P.; Biftu, T.; Parsons, W. H.; Berger, G. D. *Tetrahedron Lett.* **1993**, *34*, 6863. b) Kuo, C. H.; Robichaud, A. J.; Rew, D. J.; Bergstrom, J. D.; Berger, G. D. *BioMed. Chem. Lett.* **1994**, *4*, 1591. c) Cox, B.; Hutson, J. L.; Keeling, S. E.; Kirk, B. E.; Srikantha, A. R. P.; Watson, N. S. *BioMed. Chem. Lett.* **1994**, *4*, 1931.

(15) a) Watson, N. S.; Bell, R.; Chan, C.; Cox, B.; Hutson, J. L.; Keeling, S. E.; Kirk, B. E.; Procopiou, P. A.; Steeples, I. P.; Widdowson, J. *BioMed. Chem. Lett.* **1993**, *3*, 2541. b) Biftu, T.; Acton, J. J.; Berger, G. D.; Bergstrom, J. D.; Dufresne, C.; Kurtz, M. M.; Marquis, R. W.; Parsons, W. H.; Rew, D. R.; Wilson, K. E. *J. Med. Chem.* **1994**, *37*, 421.

(16) Chiang, Y. P.; Biftu, T.; Doss, G. A.; Plevyak, S. P.; Marquis, R. W.; Bergstrom, J. D.; Kurtz, M. M.; Rew, D. J.; Berger, G. D. *BioMed. Chem. Lett.* **1993**, *3*, 2029.

(17) a) Chan, C.; Inglis, G. G. A.; Procopiou, P. A.; Ross, B. C.; Srikantha, A. R. P.; Watson, N. S. *Tetrahedron Lett.* **1993**, *34*, 6143. b) Lester, M. G.; Giblin, G. M. P.; Inglis, G. G. A.; Procopiou, P. A.; Ross, B. C.; Watson, N. S. *Tetrahedron Lett.* **1993**, *34*, 4357.

(18) Giblin, G. M. P.; Bell, R.; Hancock, A. P.; Hartley, C. D.; Inglis, G. G. A.; Payne, J. J.; Procopiou, P. A.; Shingler, A. H.; Smith, C.; Spooner, S.J. *BioMed. Chem. Lett.* **1993**, *3*, 2605.

(19) Procopiou, P. A.; Bailey, E. J.; Bamford, M. J.; Craven, A. P.; Dymock, B. W.; Houston, J. G.; Hutson, J. L.; Kirk, B. E.; McCarthy, A. D.; Sareen, M.; Scicinski, J. J.; Sharratt, P. J.; Snowden, M. A.; Watson, N. S.; Williams, R. J. *J. Med. Chem.* **1994**, *37*, 3274.

(20) a) Abdel-Rahman, H.; Adams, J. P.; Boyes, A. L.; Kelly, M. J.; Mansfield, D. J.; Procopiou, P. A.; Roberts, S. M.; Slee, D. H.; Watson, N. S. *J. Chem. Soc. Chem. Comm.* **1993**, 1839 and 1841. b) Abdel-Rahman, H.; Adams, J. P.; Boyes, A. L.; Kelly, M. J.; Mansfield, D. J.; Procopiou, P. A.; Roberts, S. M.; Slee, D. H.; Watson, N. S. *J. Chem. Soc. Perkin Tran. I* **1994**, 1259. c) Gurjar, M. K.; Das, S. K.; Saha, U. K.; *Tetrahedron Lett.* **1994**, *35*, 2241.

(21) McVinish, L. M.; Rizzacasa, M. A. *Tetrahedron Lett.* **1994**, *35*, 923.

(22) Aggarwal, V. K.; Wang, F. M.; Zaparucha, A. *J. Chem. Soc.* **1994**, *1*, 87.

(23) Evans, D. A.; Robichaud, A. J.; Berger, G. D. *Tetrahedron Lett.* **1993**, *34*, 8403.

DETERMINATION OF REACTION PATHWAY COORDINATES BY STRUCTURE CORRELATION ANALYSIS OF CRYSTAL STRUCTURES

Reported by Peng Zhang November, 14, 1994

INTRODUCTION

One of the inherent difficulties in investigating the mechanism of chemical reactions is the lack of information about the geometrical changes along the reaction pathway. The structure of the transition state can only be determined indirectly from kinetic and spectroscopy data.[1] Structure correlation analysis (SCA) can derive the reaction coordinates and afford direct structural information about transition states from crystal structures. Group theoretical and statistical techniques are important tools in the data analysis. Since it was first proposed twenty years ago[2], structure correlation analysis has been applied to a variety of chemical transformations of organic, inorganic, organometallic, and cluster compounds. One major factor has been the dramatic expansion of crystallographic data over last twenty years. The coordinate data for organic and organometallic molecules are stored in computer-readable form in the Cambridge Structural Database (CSD). This abstract will review the background, methodology, and major applications of SCA in the mechanistic investigation of organic chemical reaction pathways.

STRUCTURE CORRELATION ANALYSIS METHOD

Although a single molecular structure cannot reveal much information about a reaction pathway, a sufficiently large number of crystal structures containing the molecular fragment of interest may be considered as a series of "photographic snapshots" taken along the reaction coordinate if they are arranged in the correct order. Thus, the structure correlation method allows one to "visualize" the geometrical changes of the fragment as the reaction proceeds from initial to final states.

How can crystal structures be related to chemical reactivity? Interatomic distances can be used as a rough measurement. Distances between two iodine atoms in various triiodide anions covers the range from I-I covalent bond length to van der Waals separation continuously.[3] Figure 1 (a) shows the changes of interatomic distances in a series of triiodide anions and figure 1 (b) shows the scatter plot of interatomic distances.The distribution of the crystal structures is far from random and indicates a strong correlation between two interatomic distances.[4] The correlation curve is used to

Copyright © 1994 by Peng Zhang

describe the coordinate of the reaction: $I_1I_2 + I_3^- \longrightarrow I_1^- + I_2I_3$. Similar correlations have been found in other linear triatomic structures, e.g. O···H-O[5], S ···S-S[4], Mo···O-Mo[6]. All analytical formulas used to represent the correlations may be obtained from a logarithmic relationship between interatomic distance (d) and bond number (n) proposed by Pauling[7]:

$$d(n) = d(1)\text{-}c \log n.$$

The constant c was assigned different values for different systems.[8] The correlation curve is similar to the minimum energy pathway on the calculated potential energy surface for the reaction: $H_2 + H \longrightarrow H + H_2$.[9]

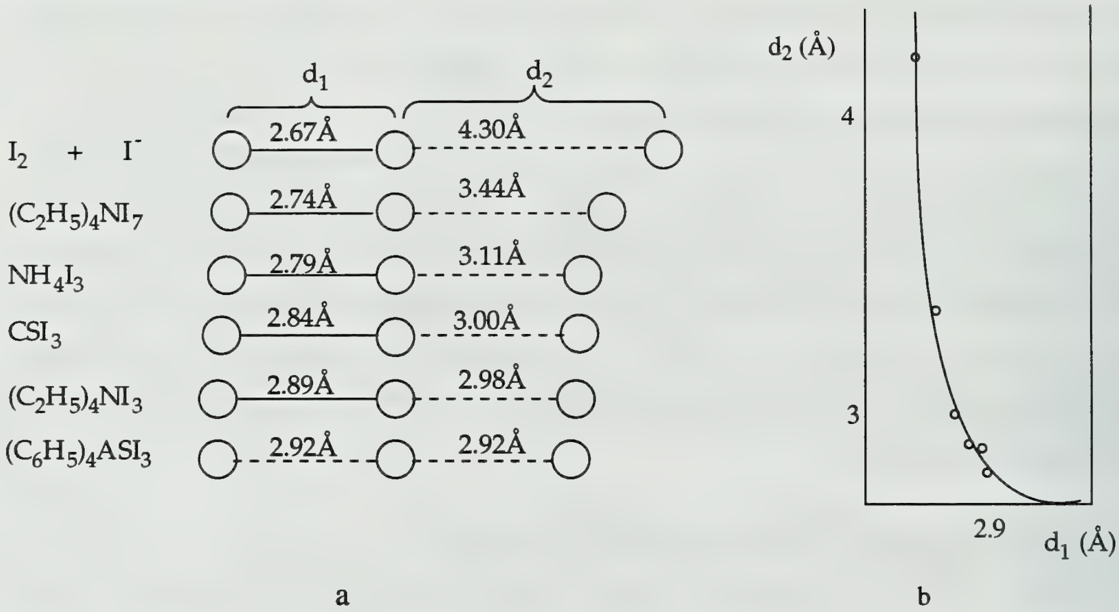


Fig. 1 (a) Interatomic distances in linear triiodides; (b) Correlation plot of interatomic distances in linear triiodide anion.

Assumptions in Structure--Structure Correlation

A basic assumption underlying SCA is that the observed crystal structures which represent the stable atomic arrangements will tend to be concentrated in low-lying regions of the potential energy surface. Therefore these related crystal structures may be represented by a series of points along the pathway of minimum energy which corresponds to the chemical reaction. Implicit in the correlation is the assumption that related molecules have similar potential energy surfaces which can be treated as various deformations from a reference fragment.[10] The deformations (i.e. changes in bond lengths, non-bonded distances, bond angles, torsional angles and dihedral angles), come from the molecular structure itself and the influence of the force field from the crystal environment. A quantitative relationship between energy and the distribution of datapoints cannot be derived from statistical analysis alone owing to the lack of information of force constant.[11]

Cambridge Structural Database (CSD): Source of SCA

CSD currently contained 120,481 entries on March, 3, 1994. The software for search, retrieval, display, and analysis of retrieved data is now available. Program QUEST3D, GSTAT, and PLUTO are used for defining and then searching the fragment of interest, for statistical analysis of the data and for viewing the crystal structures in 3-dimensions respectively.[12]

Data Analysis: Statistical Techniques

Data analysis is done in two stages. The first step is to analyze single geometrical parameters or pairs of them to determine whether the variance is caused by structural factors or experimental error. The second step consists of mapping the data in a space spanned by the geometrical parameters and finding the correlation between the parameters. For a molecule containing N atoms, there are 3N-6 independent parameters. Thus multivariate statistical methods[13] are used to reduce the dimensionality. Principle component analysis (PCA)[14], one of the most frequently used techniques, can generate a new set of linear combination of parameters, principle components, or PCs in decreased order of importance. The percentage of the total variance is the largest for the first PC, second for the second PC, and so on. If the first k (k<3N-6) PCs account for most (e.g. >90%) of the data variance, the original 3N-6 dimensional space is replaced by k-dimensional PC-space.

GEOMETRICAL CHANGES ALONG REACTION COORDINATES

Nucleophilic Additions and Eliminations at the Carbonyl Group

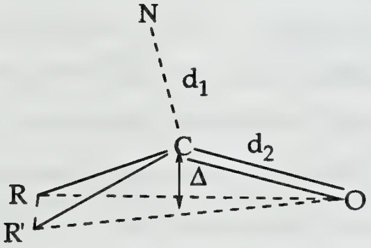


Fig. 3 Definition of geometrical parameters describing the disposition of nucleophile N relative to the carbonyl group

A prominent achievement of SCA concerns the trajectory of nucleophilic addition to carbonyl groups. Transannular interactions of N···C=O in medium ring of amino ketones had been investigated for a long time[15] when in 1973 Bürgi and Dunitz examined six crystal structures containing this fragment by the SCA method.[16] Structural parameters for the fragment are shown in Figure 3. The results reveal the geometric changes in the initial stage of nucleophilic attack to the carbonyl group. Figure 4 shows the correlation curve of Δ, which represents the pyramidalization of the carbonyl group against N···C distances (d_1). Again, the correlation is represented by a logarithmic relationship:

$$d_1 = -1.701 \log\Delta + 0.867Å$$

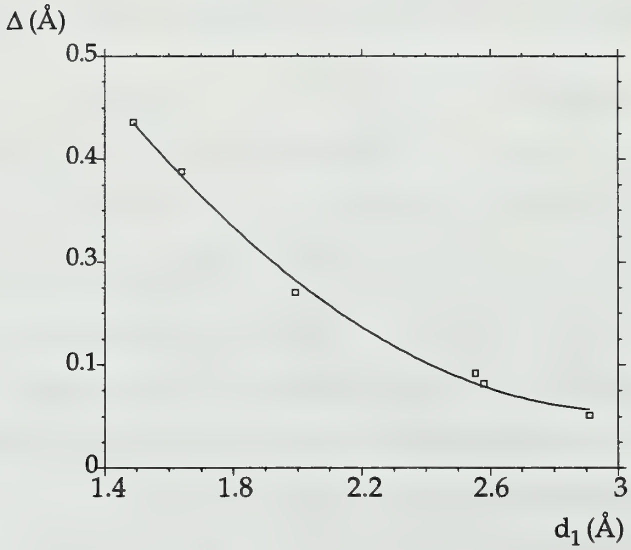
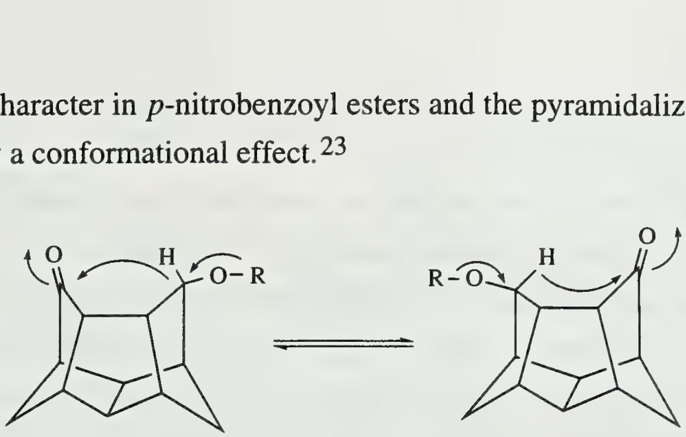


Fig. 4 Correlation plot of pyramidalization Δ vs. N···CO distance d_1[16]

Moreover, the N atoms lie close to the plane bisecting the carbonyl group and the N···C=O angles remain approximately constant at 107°. This suggests that a non-perpendicular approach of nucleophiles is preferred which is described as the "rearside" model.[17] Subsequent SCA on intra and intermolecular N···C=O and O···C=O interactions by structural correlation analysis were generally consistent with the initial description of the nucleophilic attack angle.[18,19] But there was some controversy about the pyramidalization of carbonyl groups. For O···C=O interactions, the data show considerable scatter and Δ values are less than those observed for N···C=O at similar d_1 values.[20] The lower Δ values are attributed to the weaker nucleophilicity of oxygen in comparison to that of nitrogen. However, later data show that a similar correlation curve for N···C=O interaction lies below the original curve. It is postulated that in some cases the carbonyl oxygen may act as nucleophile to form secondary nucleophile-electrophile interactions, such as C=O···C=O, and C-H···O interactions, which offset the O···C=O interaction and decrease the Δ values.[21] Crystal structures of polycyclic hydroxy ketones, especially compound **I** show the pyramidalization of the carbonyl group toward the hydrogen. It is viewed as the initial stage of the intramolecular hydride transfer reaction (eq. 1).[22] Cieplak argues that the carbinol hydrogen is expected to show little nucleophilic character in *p*-nitrobenzoyl esters and the pyramidalization in compound **I** is most probably a conformational effect.[23]



(1)

I,R = *p*-$NO_2C_6H_4CO$

The most important support for the non-perpendicular approach model came from *ab initio* calculation, which has reproduced the correlation curve of pyramidalization of the carbonyl group (Δ) against the incipient bond length d_1 for hydride addition to formaldehyde and the approximate constancy of attack angle (100-110°).[24]

The tetrahedral acetal fragment is the product of completed O···C=O addition. Crystal structures of acetals are considered to map out the final stage of nucleophilic addition to carbonyl groups and the initial stage of the elimination reaction. The correlations reveal that the antisymmetric stretching couples with bending along the reaction coordinate. The leaving group does not depart along the direction of C-O bond. Figure 5 shows that as the shortening C-O bond moves toward the R-C-R' plane whereas the direction of departure of the leaving group is continuously adjusted to maintain the angle O-C-O around 110°. This is interpreted in terms of a preferred direction of approach of the nucleophile to the carbonyl group.[20] Later research showed a considerable variation of O-C-O angles which are about 80-90° instead of 100-110° when anomeric effects are involved.[31]

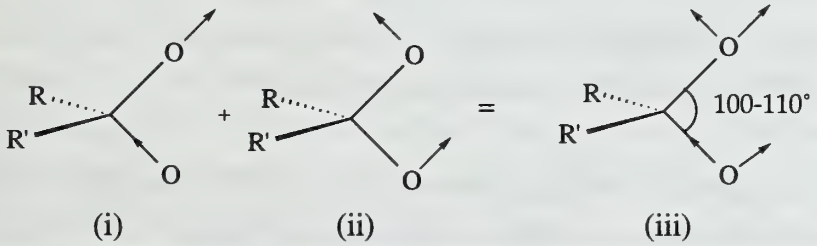


Fig. 5 Vibrational mode in the process of elimination of acetals (i) antisymmetric stretch; (ii) bend; (iii) the coupling of (i) and (ii)

Structure correlation analysis has been extended to an alternative type of addition/elimination at carbonyl groups via an acylium intermediate.[26] Because only limited crystallographic data for such fragment are available, the bond length and angles obtained from other techniques are used in the correlation.

"Rearside" Attack Model in Stereochemistry of Nucleophilic addition to Carbonyl Groups

The non-perpendicular approach model has been extended to other unsaturated functional groups like C=C double and triple bonds. It is a basic assumption in Baldwin's vector analysis and rules for ring closure; in Liotta's and Burgess's trajectory analysis, and in various models of asymmetric induction by Anh, Houk, and Heathcock; and in explanations for aldol diastereoselectivity by Seebach, Anh, and Eschenmoser.[27] The "rearside" attack model has also been used to explain the high regio- and diastereoselectivity in metal hydride reductions of *gem*--disubstituted succinimides and succinic anhydrides.[17]

Nucleophilic Substitution Reactions at Tetrahedral Molecules

The tetrahedral fragments with approximate C_{3v} symmetry are considered to map the coordinate of the S_N1 reaction[28]: the axial bond is cleaved and planar trigonal structures are formed. The results also reveal that the correlation curves are indistinguishable for different fragments.

SCA on pentacoordinate Cd compounds reveal an S_N2 type reaction coordinate (eq. 2):[2]

$$Y + CdS_3X \longrightarrow [Y\cdots CdS_3\cdots X] \longrightarrow YCdS_3 + X \qquad (2)$$

where X,Y=I, O, S. The transition states derived from the correlation curve corresponds to trigonal bipyramidal structure with D_{3h} symmetry. Later work on Sn, Pb Si, Ge shows similar correlations.[30]

Correlation studies for six-coordinate complexes ($SnR_2X_2Y_2$) reveal a possible mechanism for a termolecular double substitution reaction (eq. 3):

$$SnR_2X_2 + 2Y \longrightarrow [SnR_2X_2Y_2] \longrightarrow SnR_2Y_2 + 2X \qquad (3)$$

which is dubbed the S_N3 reaction by Dunitz.[29] No experimental observation of such an entropically disfavored reaction has been found up to now.

STRUCTURE--ENERGY CORRELATION: REACTION PROFILES FROM SCA

Although there is no quantitative relationship between crystal structures and the energy, the potential energy surface and the structure of transition state may be derived by combining SCA with activation energy data. A successful example is the symmetrical degenerate ring inversion of (*s-cis*-η^4-butadiene)metallocene complexes.[25] Figure 6 shows the definition of the parameters and the ring inversion reaction. Principle component analysis reveals two major PCs. The first PC corresponds to the ring inversion coordinate which can be described by the dihedral angle θ and the second PC represents the bite size which can be described by the distances of $CH_2\cdots CH_2$ (d).

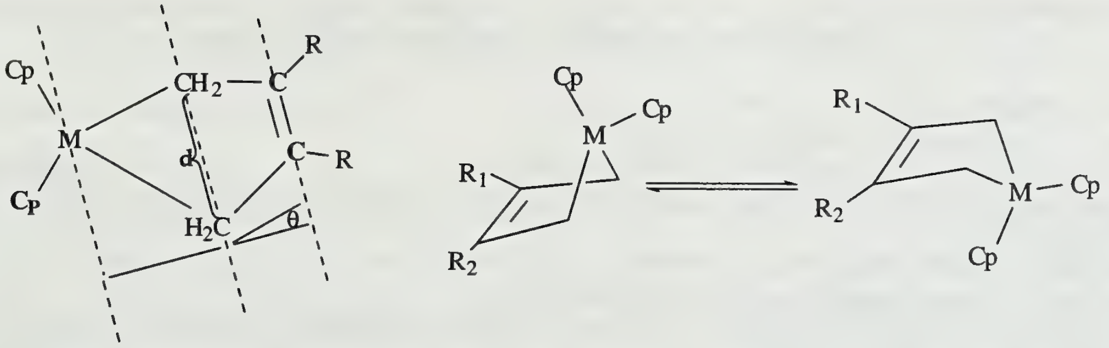


Figure 6 Definition of parameters and the ring inversion reaction.

The correlation curve of two PCs is approximately a symmetrical semicircle and the middle top point represents the transition state structure which is planar. The distances between the data points and the transition state are correlated with the measured activation energy of each molecule by a fourth order equation and the reaction profile expression is then derived from it.

The same methodology has been applied to nondegenerate reactions, e.g. hydrolysis of axial tetrahydropyranyl acetals.[25] An expression for the potential energy of the reaction has been proposed and the structures of the transition state calculated are compatible with the one derived from the structure correlation analysis.

Structure correlation analysis can describe the trajectory of the reaction but there is no direct link with the potential energy surface. However, in combination with other techniques, e.g. kinetic measurements, energy surface and the structure of transition state can be constructed. The method sometimes is restricted by the lack of either structural or kinetic data.

CONCLUSIONS AND FUTURE WORK

Structure correlation method affords important information and insight about reaction coordinates, e.g. the detailed structural changes necessary to deform the ground state of the reactants into the transition state and characteristics of the transition state itself. However, several limitations should be kept in mind. First, only a qualitative picture of the potential energy surface can be drawn from SCA, and it cannot distinguish between one-step and multi-step mechanisms; Second, the results depend greatly on the choice and treatment of crystallographic data; Third, when the structures become complicated the greater number of parameters involved and the more complicated geometric relations impede the statistical analyses. Future work will focus on the combination of SCA with kinetic, mechanistic, and computational approaches to understand the structural and energy changes that take place during dynamic chemical transformations.

REFERENCES

1 For example, the endocyclic restriction test: Beak, P. *Acc. Chem. Res.* **1992**, *25*, 215-222

2 Bürgi, H.B. *Inorg. Chem.* **1973**. *12,* 2321-2325

3 Bent, H.A. *Chem. Rev.* **1968,** *68*, 587-648

4 Bürgi, H.B. *Angew. Chem., Int. Ed.* **1975**, *14*, 460-473

5 Hamilton, W.C.; Ibers, J.A. *Hydrogen Bonding in Solids*; W.A. Benjamin: New York, 1968, p 54

6 Kihlborg, L. *Ark. Kemi,* **1963**, *21*, 471

7 Pauling, L. *J. Am. Chem. Soc.*, **1947**, *69*, 542-553

8 Dunitz, J.D. *X-ray Analysis and the Structure of Organic Molecules;* Cornell: London, 1979, pp 344-346.

9 Liu, B. *J. Chem. Phys.* **1973**, *58,* 1925

10 Bürgi, H.B., Dunitz, J. D. *Acc. Chem. Res.* **1983**, *16*, 153-161

11 Bürgi, H.B.; Dunitz, J. D. *Acta. Cryst.* **1988**, *B44*, 445-448

12 See detailed information about CSD software: *CSD System Documentation*, Vol. 1-4, Cambridge Crystallographic Data Center, Oct. **1992**

13 Chatfield, C.; Collins, A.J. *Introduction to Multivariate Analysis*, Chapman and Hall: London, 1980.

14 Heyde, T.P.E.Auf *J. Chem. Ed.,* **1990** *67,* 461

15 Leonard, N.J. *Rec. Chem. Prog.,* **1956**, *17,* 243

16 Bürgi, H.B.; Dunitz, J. D.; Shefter, E. J. *Am. Chem. Soc.*, **1973**, *95*, 5065-5067

17 Rosenfield, R.E.; Dunitz, J.D. *Helv. Chim. Acta.* **1978**, *61*, 2176-2189

18 Schweizer, W.B.; Procter, G.; Kaftory, M.; Dunitz, J.D. *Helv. Chim. Acta.* **1978**, *61*, 265-290

19 (1) Brennan, R.G.; Prive, G.G.; Blonski, W.J.P.; Hruska, F.E.; Sundaralingram, M.S. *J. Am. Chem. Soc.* **1983**, *105,* 7737-7742; (2) Spanka, G.; Boese, R.; Rademacher, P. *J. Org. Chem.* **1987**, *52,* 3362-3367

20 Bürgi, H.B.; Dunitz, J. D.; Shefter, E. *Acta Cryst.* **1974**, *B30*, 1517-1527

21 Cossu, M.; Bachmann, C.; N'Guessan, T.Y.; Viani, R., Lapasset, J.; Aycard, J-P.; Bodot, H. *J. Org. Chem.* **1987**, *52*, 5213-5319

22 Cernik, R.; Craze, G-A.; Mills, O.S.; Watt, I.; Whittleton, N. *J. Chem. Soc. Perkin II* **1984,** 685-690

23 Cieplak, A.S. In *Structure Correlation*; Burgi, H.B., Dunitz, J. D. Ed.;VCH Publishers: Weinheim, 1994, p 213-214

24 Bürgi, H.B.; Lehn, J.M.; Wlpff, G. *J. Am. Chem. Soc.* **1974,** *96*, 1956

25 Bürgi, H.B.; Dubler-Steudle K.C. *J. Am. Chem. Soc.* **1988**, *110*, 4953-4957

26 Ferretti, V.; Dubler-Steudle, K.C.; Burgi, H.B. In *Accurate Molecular Structures;* Domenicano, A.; Hargittai, I. Ed.; Oxford: London, 1992, 412-436

27 (a)Baldwin, J.E. *J. Chem. Soc. Chem. Commun.* **1976,** 738; (b) Baldwin, J.E. *J. Chem. Soc. Chem. Commun.* **1976**, 734; (c) Liotta, C.S.; Burgess, E.M.; Ebeerhardt, W.H. *J. Am. Chem. Soc.* **1984**, *106*, 4849; (d) Anh, N.T.; Eisenstein, O. *Nouv. J. Chim.* **1977**, *1*, 61; (e) Houk, K.N. et al. *Science*, **1986**, *231*, 1108; (f) Heathcock, C.H.; Flippin, L.A.; *J. Am. Chem. Soc.* **1983**, *105*, 1667; (g) Seebach, D.; Golinski, J. *Helv. Chim. Acta.* **1981**, *64*, 1413; (h) Anh, N.J.; Thanh, B.T. *Nouv. J. Chim.* 1986, 10, 681; (i) Muller, D.' Pitsch, S.; Kittaka, A.; Wagner, E.; Wintner, C.E.; Eschenmoser, A. *Helv. Chim. Acta.* **1990**, *73*, 1410

28 Murray-Rust, P., Bürgi, H.B., Dunitz, J.D. *J. Am. Chem. Soc.*, **1975**, *97,* 921

29 Britton, D., Dunitz, J.D. *J. Am. Chem. Soc.*, **1981**, *103*, 2971-2979

30 Bürgi, H.B.; Shklover, V. In *Structure Correlation*; Burgi, H.B., Dunitz, J. D. Ed.; VCH Publishers: Weinheim, 1994, p 168-169

31 Bürgi, H.B.; Dubler-Steudle K.C. *J. Am. Chem. Soc.* **1988**, *110*, 7291-7299

DEVELOPMENTS IN THE OSMIUM TETROXIDE CATALYZED ASYMMETRIC DIHYDROXYLATION OF OLEFINS

Reported by James C. Nelson December 5, 1994

INTRODUCTION

Remarkable progress has been made in the catalytic asymmetric *cis*-1,2-dihydroxylation of olefins by OsO_4 and chiral amine ligands in the past five years. The cinchona alkaloid based catalysts developed by Sharpless are especially practical owing to high catalytic turnover, high yields, high enantioselectivities and the wide range of substrates which may be selectively oxidized.[1] Recent advances make this reaction one of the most general enantioselective processes known. Although several proposals have been formulated, the mechanism and origin of stereoselectivity are still in dispute. This report, a continuation of Anantharaman's, summarizes advances in this widely used reaction, since 1990.[2]

BACKGROUND

Criegee et al. first observed in 1942 that pyridine and certain tertiary amines accelerate the addition of OsO_4 to olefins.[3] This observation presented the possibility of using a chiral amine to influence the enantioselectivity of the osmylation to produce chiral 1,2-diols. Sharpless et al. in 1980 used acetate derivatives of the diastereomeric cinchona alkaloids, dihydroquinidine (DHQD) and dihydroquinine (DHQ), in the osmylation of *(E)*-disubstituted olefins to produce enantiomerically enriched vicinal diols.[4a] These two alkaloid derivatives gave opposite stereoselectivities, with dihydroquinine acetate affording the *(S,S)*-diol and dihydroquinidine acetate affording the *(R,R)*-diol. Shortly following, several other workers (Narasaka and Yamada (1986), Tokles and Snyder (1986), Tomioka et al. (1987), Hirama and Oishi (1989), and Corey et al. (1989)) presented similar stoichiometric asymmetric dihydroxylations based on the use of chiral amines and diamines.[2] The stoichiometric asymmetric osmylations, notably those employing chiral diamines, afford *cis*-1,2-diols with good to high enantiomeric excess. Disadvantages were their limitation to *trans*-1,2-disubstituted olefins and the requirement of a stoichiometric amount of OsO_4, which is costly and highly toxic.

The high cost and toxicity of OsO_4, provided incentive for the development of a catalytic asymmetric osmylation process, particularly for large-scale preparations of optically active *cis*-1,2-diols. Sharpless et al. first reported a novel catalytic asymmetric osmylation in 1988, employing aryl ester derivatives of the previously mentioned cinchona alkaloids and *N*-methylmorpholine *N*-oxide as the secondary oxidant.[5] This gave good diol yields with moderate enantioselectivity. A two cycle catalytic mechanism was proposed for this process, one resulting in high enantioselectivity and the other which resulted in low enantioselectivity.[6] Evidence for this two

Copyright © 1994 by James C. Nelson

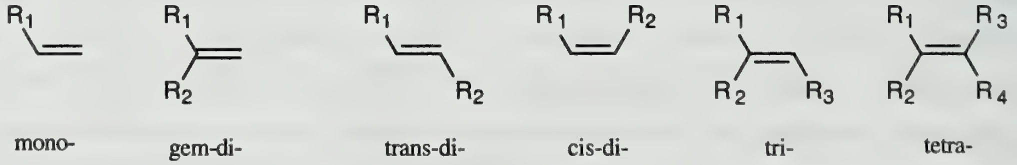
cycle proposal was provided by carrying out the process in a stepwise manner under stoichiometric conditions. Sharpless et al., based on their two cycle mechanism, were able to suppress the low enantioselective cycle by slow addition of olefin. This modification resulted in enantioselectivities comparable to the stoichiometric process with only 0.2-0.4% of OsO$_4$ required for the transformation. Later, by changing the stoichiometric oxidant to K$_3$Fe(CN)$_6$ and changing the solvent from acetone/water to *tert*-butyl alcohol/water, higher enantioselectivity was effected without the need for slow addition of olefin.[1] This observation was attributed to complete suppression of the low enantioselective cycle in their proposed mechanism. This interpretation was substantiated by evidence showing that hydrolysis precedes re-oxidation in the catalytic cycle, thus precluding the possibility of the low enantioselective cycle.

In addition to modifications to the catalytic process involving the stoichiometric oxidant and solvent, Sharpless et al. made several other important early observations which have guided the development of this reaction. It was shown that the amount of osmium used could further be reduced by using a polymer bound osmium catalyst which could be reused.[7] X-ray structural and NMR conformational studies showed that the cinchona alkaloid ligands used changed conformation upon complexation with OsO$_4$[8] and, most importantly, changes in the substituents on the cinchona alkaloid ligand affect the enantioselectivity of the reaction[9] (i.e. changing the 9-*O*-substituent on the cinchona alkaloid ligand to *p*-chlorobenzoyl (CLB) (**1a**, **2a** in Scheme II) resulted in improved enantioselectivity).

LIGAND MODIFICATION, EXPANDS THE SCOPE OF REACTION

At the time of Anantharaman's[2] report in 1990, the catalytic asymmetric dihydroxylation reaction was experiencing high yields and high enantioselectivity with synthetic application to simple hydrocarbon olefins, aromatic olefins, allylic alcohols and their esters, allylic chlorides, α,β-unsaturated aldehydes, ketals of α,β-unsaturated ketones and water-sensitive trimethylsilyl enol ethers. However, the early catalytic process only resulted in useful enantioselectivity for two out of six possible prochiral olefin classes (Scheme I), namely trans-di-, tri- and to a lesser extent mono- and gem-di- substituted olefins. It was clear that this reaction showed potential for improvement through ligand modification and/or a better understanding of the osmylation mechanism.

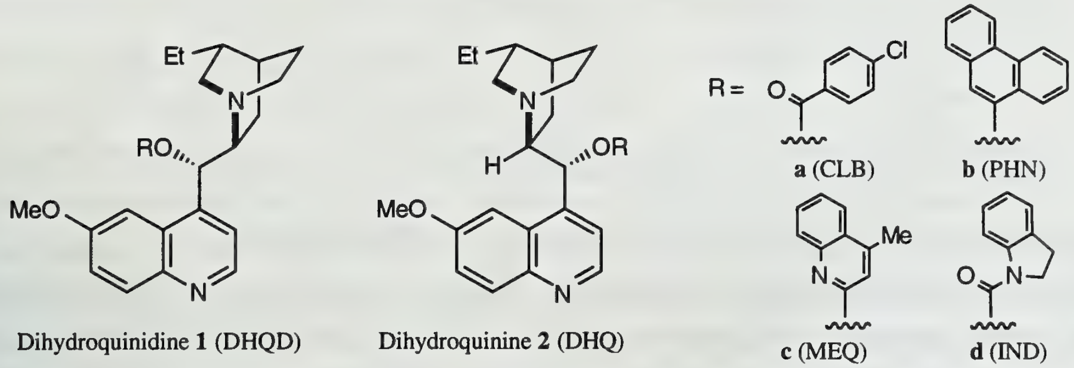
Scheme I



Sharpless et al. in 1991 introduced a ligand modification in which the cinchona alkaloid ligand was modified by changing the substituent of the 9-*O*- position from CLB to 9-phenanthryl

(PHN) (**1b**, **2b**) and 4-methyl-2-quinolyl (MEQ) (**1c**, **2c**) as shown in Scheme II.[10] These seemingly small changes in ligand resulted in improved enantioselectivity for terminal olefins. For example a 93% ee was reported for vinylcyclooctane (a previously poor substrate). Shortly thereafter, the enantioselectivity was further improved by changing the 9-*O*- substituent to 1-naphthyl.[11]

Scheme II



Dihydroquinidine **1** (DHQD)

Dihydroquinine **2** (DHQ)

a (CLB)

b (PHN)

c (MEQ)

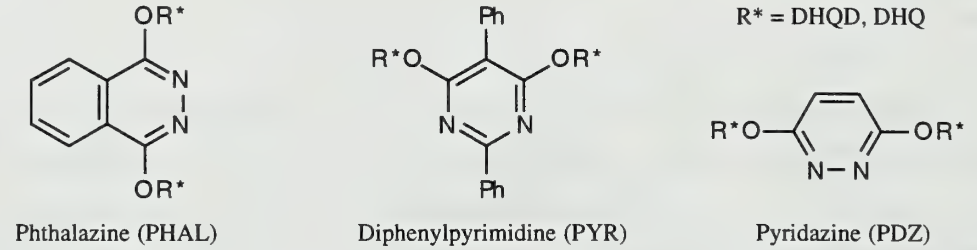
d (IND)

The largest expansion in the scope of this reaction came in 1992, when Sharpless et al. introduced a new class of bis-cinchona alkaloids with a 1,4-phthalazine (PHAL) spacer unit as shown in Scheme III.[12] In addition, the use of methylsulfonamide was introduced to aid in the hydrolysis of the osmate ester . Sharpless commercialized powdered mixtures of the following reagents: $(DHQD)_2PHAL$ or $(DHQ)_2PHAL$ ligands, $K_2OsO_2(OH)_4$, $K_3Fe(CN)_6$ and K_2CO_3, which are sold under the trade names AD-mix-α and AD-mix-β, respectively. Because of the accelerated rate of osmate ester hydrolysis by the added sulfonamide, most reactions could be run at 0 °C with reaction times of 6-24 h. These changes, in addition to being safe and practical (since $K_2Os(OH)_4$ is non-volatile and AD-mix-α and AD-mix-β can be used for reactions up to 5 mmol in olefin), gave exceptional enantioselectivity making the catalytic asymmetric dihydroxylation general, efficient and highly enantioselective.

Following the discovery of the bis-cinchona alkaloid class of ligands and their observed enantioselectivity enhancement, Sharpless et al. developed other ligands based on the same empirical methodology of 9-*O*-substituent modification.[13] Modification to indolinylcarbamoyl (**1d**, **2d**) (Scheme II), resulted in improved enantioselectivity for the *cis*-di-substituted class of olefins.[13a] Changing the PHAL spacer to 4,6-(2,5-diphenylpyrimidine) (PYR) (Scheme III) enhanced the enantioselectivity for terminal olefins.[13b] It was observed that the PHAL and PYR ligands gave modest to high enantioselectivity for some tetra-substituted olefins.[13c] Corey et al. introduced a 3,6-pyridazine (PDZ) (Scheme III) ligand which gave enantioselectivities similar to those obtained with the Sharpless PHAL ligand.[14a]

Since 1988, the catalytic asymmetric dihydroxylation of olefins has experienced dramatic improvement. These modifications have expanded the synthetic application to include representatives of every class of prochiral olefins, kinetic resolution of racemic olefins, and difficult oxidations[15] such as olefins containing sulfides, 1,3-dithianes, and disulfides. The wide use of this reaction is documented by numerous citations in the recent literature, as well as its application in many total syntheses in the past year alone.

Scheme III



R* = DHQD, DHQ

Phthalazine (PHAL) Diphenylpyrimidine (PYR) Pyridazine (PDZ)

INVESTIGATIONS ON THE ELUCIDATION OF MECHANISM

Despite the wide usage of the asymmetric dihydroxylation process, several mechanistic questions remain unresolved. Currently two mechanisms for the osmylation of olefins are being considered: a concerted [3+2] cycloaddition mechanism (Figure 1, path A) and a stepwise mechanism which involves the formation and subsequent rearrangement of an osmaoxetane intermediate (path B), formerly the product of a [2+2] cycloaddition. The primary concern of both mechanistic proposals is the origin of stereoselectivity. These mechanistic questions as well as others have been addressed via X-ray structural and NMR conformational analysis, ligand modification correlations, reaction kinetics and a variety of computational investigations.

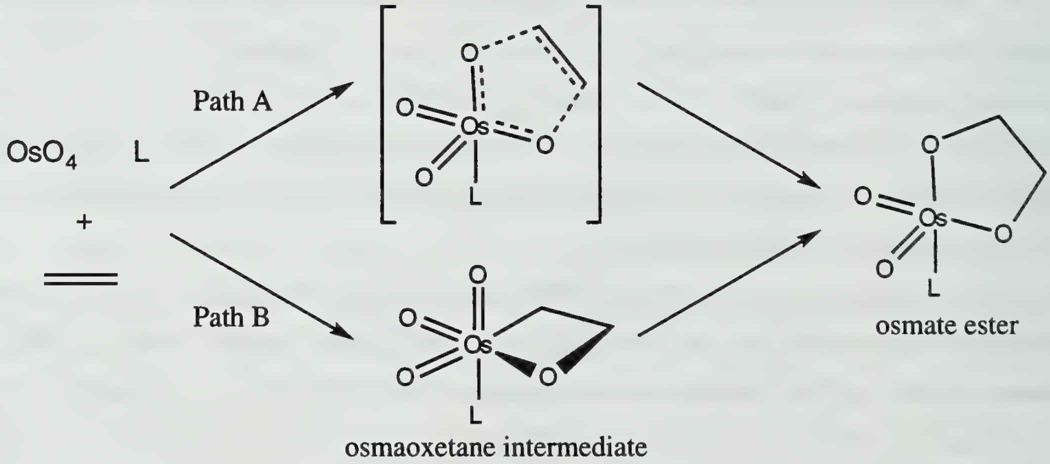


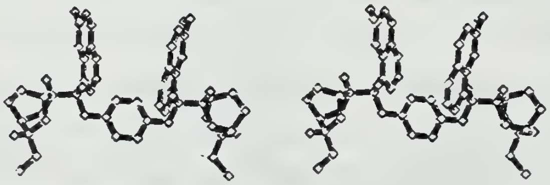
Figure 1. Schematic representation of the concerted [3+2] mechanism (path A) and the stepwise osmaoxetane mechanism (path B) for the asymmetric dihydroxylation of olefins by OsO_4.

[3+2] Concerted Mechanism: Criegee et al. first implied the [3+2] osmylation mechanism in 1942.[3] Corey et al. in 1989 proposed a [3+2] mechanism to explain the observed stereoselectivity for their 1,2-diphenyl-1,2-diaminoethane ligand used in the stoichiometric dihydroxylation.[16] The stereochemical course of osmylation was rationalized via a bidentate octahedral complex of the amine ligand and OsO_4 as the reactive species. An unsymmetrical transition structure was envisioned, involving one equatorial oxygen and one axial oxygen from the osmium ligand complex. Houk et al., based on modified MM2 calculations, later proposed a symmetrical attack by the osmium complex involving both of the equatorial oxygens.[17] Both the Corey and Houk models are capable of explaining observed facial selectivities, but only the latter is consistent with the high selectivity found for *trans*-stilbene and methylstyrene.

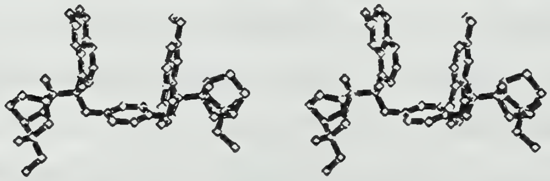
While these original models explain the origin of stereoselectivity in the stoichiometric process, they do not do so for the catalytic process. This mechanistic dichotomy arises from the differential affinity of OsO_4 for amine ligands.[18] Kinetic data show second order behavior for pyridine; however, the cinchona alkaloid ligands show either first order or saturation rate behavior, which are both consistent with a 1:1 ligand : osmium complex as the reactive species. The Corey and Houk models do not explain the observed enantioselectivity for the catalytic process, which does not arise from a bidentate osmium complex. This dilemma prompted Corey et al. to propose an alternative [3+2] model based on a μ-oxo-bridged bis-Os(VIII) complex as the reactive species.[14a, b] This model was supported by the existence of the related μ-oxo-bridged species MoO_2Cl_2, a reasonable hypothetical structure accounting for enantioselectivity and a ligand modification indicating that both alkaloid units of the bis-cinchona alkaloid ligand were essential for high enantioselectivity.[14b]

The μ-oxo-bridged bis-Os(VIII) model, although considered reasonable by Veldkamp and Frenking[19] based on ab initio calculations, was soon refuted by Lohray et al.[20] and Sharpless et al.[21] Lohray's argument was based on the observed reactivity of three modified bis-cinchona alkaloid ligands with varying spacer lengths. With a spacer allowing only for chelation and not formation of the μ-oxo-bridged species, the reaction did not proceed catalytically. When the spacer was lengthened, allowing for formation of the μ-oxo-bridged species, high enantioselctivity was observed (ca. 90% ee), with the reaction proceeding catalytically. When a spacer was used which disfavored the μ-oxo-bridged species, the reaction exhibited even higher enantioselctivity (> 98% ee). Lohray argued that this result indicated that the two alkaloid units of the bis-cinchona alkaloid ligand act independently and not via the μ-oxo-bridged species. Sharpless' arguments were based on previously established kinetics studies (showing first order rate behavior in OsO_4) and a series of ligand modifications clearly showing, that only one alkaloid unit in the bis-cinchona alkaloid is essential for high enantioselctivity. Later Corey et al. ruled out their μ-oxo-bridged model in 1993, based solely on their synthesis of a conformationally restricted ligand which exhibited high enantioselectivity, yet could not form the μ-oxo-bridged species.[22a]

In connection with the dismissal of the μ-oxo-bridged model, Corey et al. proposed another [3+2] based model, this time invoking the existence of a 'U-shaped' enzyme binding pocket, to account for the stereoselectivity (Figure 2).[22a] This model was based on X-ray structural data of methiodide salts of the conformationally restricted ligand (mentioned above) and the PDZ ligand, as well as NMR conformational studies of methiodide salts and OsO_4 complexed ligands, all of which indicated that the ligands adopt a 'U-shaped' conformation when complexed, but not when free. In support of their 'U-shaped' model, an unsymmetrical ligand was synthesized in which one of the alkaloid groups was replaced with a (*t*-butyl-1-anthraceylmethyl) ether moiety.[22b, c] NMR conformational studies indicated again a 'U-shaped' conformation for the complexed ligand, but not for the free ligand. In addition, CHARM force field calculations revealed the 'U-shaped' conformation as a stable form for this unsymmetrical ligand.



'L-Shaped' Conformation



'U-Shaped' Conformation

Figure 2. Stereoviews of the Corey 'U-shaped' and the Sharpless 'L-shaped' conformation. Note should be made as to the position of the pyridazine spacer unit, which if tilted by 90°, interconverts the two conformers.

Sharpless et al. have refuted Corey's 'U-shaped' model, primarily based on the position of the pyridazine ring.[23] MM2 calculations conducted by Sharpless indicate that the 'U-shaped' conformation, for the PDZ ligand, is of such high energy as to be virtually inaccessible in the transition state. He claimed that this observation was intuitive, since the electron deficient pyridazine ring would clearly prefer to be planar allowing for conjugation with the alkoxy groups. Thus Sharpless concluded that the 'U-shaped' model could not account for the selectivity observed with sterically demanding olefins. While the concerted [3+2] mechanism has matured over the past 44 years, it is clear that many questions concerning enantioselctivity in particular, need be answered.

[2+2] Stepwise Mechanism: The [2+2] stepwise mechanism was first proposed by Sharpless et al. in 1976.[4a, b] While this mechanism has not been ruled out over the past 18 years, acceptance for this mechanism has been slow due to lack of direct evidence for the proposed osmaoxetane intermediate.

Among the most compelling of proof for a stepwise mechanism, was provided by Sharpless et al. in 1993, when they reported the influence of reaction temperature on the enantioselctivity of the asymmetric dihydroxylation.[24] The experiments were performed stoichiometrically in OsO_4, over a temperature range of 100°C. The Eyring plot for each olefin/catalyst system examined showed, an inversion temperature. These inversion temperatures have already been observed for other catalytic asymmetric systems, and can be explained by a reaction pathway which involves at least two enantiodetermining steps. These results are therefore consistent with the proposed [2+2] stepwise mechanism. It should be noted, that these results do not favor any particular stepwise mechanism, however, the [2+2] mechanism is the only currently proposed stepwise mechanism.

It has been shown by Sharpless et al., by using DFT (Density Functional Theory) and MM2 calculations that the osmaoxetane intermediate is plausible energetically, and that it can be formed exothermically from OsO_4-ligand and olefin or osmaoxetane and ligand (Figure 1).[25] Nevertheless, the free energy of the osmaoxetane intermediate is expected to be higher than the reactants owing to a large expected entropy term, thus accounting for the instability and resulting elusiveness of the osmaoxetane intermediate. Veldkamp and Frenking, using ab initio calculations have also concluded that the osmaoxetane intermediate is favored, albeit higher in energy then the reactants, again explaining the instability of the osmaoxetane.[19]

Sharpless et al., have proposed a model in which a 'L-shaped' binding pocket is responsible for the high enantioselctivity observed in their [2+2] stepwise mechanism (Figure 2).[23, 26] This model was based on X-ray structural data, NMR conformational data and molecular mechanics calculations. Enantioselectivity arises, from simultaneous attractive face-to-face interactions between the highly polarized phthalazine floor and edge-to-face interactions between the bystander aromatic group and the osmaoxetane intermediate. Although both diastereomeric structures leading to diol formation can experience these favorable interactions, one diastereomeric osmaoxetane experiences unfavorable steric interactions, thus explaining the observed selectivity. To further test this model, a series of systematically substituted olefins were synthesized and the enantioselectivities were examined. From these results Sharpless et al. were able to rationalize the observed enantioselctivity based on an 'L-shaped' binding pocket. They argued that similar rationalizations could not be made for the 'U-shaped' binding pocket proposed by Corey. It was also noted that the 'L-shaped' model could explain selectivity in the first-generation ligands (CLB, PHN, MEQ), whereas the 'U-shaped' model could not. While all of the evidence presented is consistent with the proposed [2+2] stepwise mechanism, direct proof for the existence of the osmaoxetane intermediate is still lacking.

CONCLUSIONS

Unprecedented progress has been made in the catalytic asymmetric dihydroxylation reaction. The synthetic scope of this reaction has continued to expand over the past five years, making this into one of the most general enantioselective processes known. Although the mechanism is not entirely clear, much progress has been made. The concerted [3+2] mechanism proposed by Corey, in addition to being inconsistent with present experimental evidence mentioned, suffers from shortcomings in predicting high enantioselectivity. The [2+2] mechanism proposed by Sharpless, on the other hand, is consistent with experimental evidence, but suffers from lack of direct proof for the proposed osmaoxetane intermediate. Future work in this area will undoubtedly continue to deal with mechanistic issues and will expand further its synthetic applications.

REFERENCES

1. Kwong, H-L.; Sorato, C.; Ogino, Y.; Chen, H.; Sharpless, K.B. *Tetrahedron Lett.* **1990**, *31*, 2999.
2. Anantharaman, V. *Chem 435 Abstract* **Oct. 11, 1990**.
3. Criegee, R.; Marchand, B.; Wannowius, H. *Liebigs Ann. Chem.* **1942**, *550*, 99.
4. (a) Sharpless, K.B.; Teranishi, A.Y.; Bäckvall, J-E. *J. Am. Chem. Soc.* **1976**, *99*, 3120. (b) Hentges, S.G.; Sharpless, K.B. *J. Am. Chem. Soc.* **1980**, *102*, 4263.
5. Jacobsen, E.N.; Markó, I.; Mungall, W.S.; Schröder, G.; Sharpless, K.B. *J. Am. Chem. Soc.* **1988**, *110*, 1968.
6. Wai, J.S.M.; Markó, I.; Svendsen, J.S.; Finn, M.G.; Jacobsen, E.N.; Sharpless, K.B. *J. Am. Chem. Soc.* **1989**, *111*, 1123.
7. Kim, B.M.; Sharpless, K.B. *Tetrahedron Lett.* **1990**, *31*, 3003.
8. (a) Dijkstra, G.D.H.; Kellogg, R.M.; Wynberg, H.; Svendsen, J.S.; Markó, I.; Sharpless, K.B. *J. Am. Chem. Soc.* **1989**, *111*, 8069. (b) Svendsen, J.S.; Markó, I.; Jacobsen, E.N.; Rao, C.P.; Bott, S.; Sharpless, K.B. *J. Org. Chem.* **1989**, *54*, 2263.
9. Shibata, T.; Gilheany, D.G.; Blackburn, B.K.; Sharpless, K.B. *Tetrahedron Lett.* **1990**, *31*, 3817.
10. Sharpless, K.B.; Amberg, W.; Beller, M.; Chen, H.; Hartung, J.; Kawanami, Y.; Lübben, D.; Manoury, E.; Ogino, Y.; Shibata, T.; Ukita, T. *J. Am. Chem. Soc.* **1991**, *56*, 4585.
11. Ogino, Y.; Chen, H.; Manoury, E.; Shibata, T.; Beller, M.; Lubben, D.; Sharpless, K.B.*Tetrahedron Lett.* **1991**, *32*, 5761.
12. Sharpless, K.B.; Amberg, W.; Bennani, Y.L.; Crispino, G.A.; Hartung, J.; Jeong, K-S.; Kwong, H-L.; Morikawa, K.; Wang, Z-M.; Xu, D.; Zhang, X-L. *J. Org. Chem.* **1992**, *57*, 2768.
13. (a) Wang, L.; Sharpless, K.B. *J. Am. Chem. Soc.* **1992**, *114*, 7568. (b) Crispino, G.A.; Jeong, K-S.; Kolb, Kolb, H.C.; Wang, Z-M.; Xu, D.; Sharpless, K.B. *J. Org. Chem.* **1993**, *58*, 3785. (c) Morikawa, K.; Park, J.; Andesson, P.G.; Hashiyama, T.; Sharpless, K.B. *J. Am. Chem. Soc.* **1993**, *115*, 8463.
14. (a) Corey, E.J.; Lotto, G.I. *Tetrahedron Lett.* **1990**, *31*, 2665. (b) Corey, E.J.; Noe, M.C.; Sarshar, S *J. Am. Chem. Soc.* **1993**, *115*, 3828.
15. (a) VanNieuwenhze, M.S.; Sharpless, K.B. *J. Am. Chem. Soc.* **1993**, *115*, 7864. (b) Walsh, P.J.; Ho P.T.; King, S.B.; Sharpless, K.B. *Tetrahedron Lett.* **1994**, *35*, 5129.
16. Corey, E.J.; Jardine, P.; Virgil, S.; Yuen, P.W.; Connel, R.D. *J. Am. Chem. Soc.* **1989**, *111*, 9243.
17. Wu, Y-D.; Wang, Y.; Houk, K.N. *J. Org. Chem.* **1992**, *57*, 1362.
18. Jacobsen, E.N.; Markó, I.; France, M.B.; Svendsen, J.S.; Sharpless, K.B. *J. Am. Chem. Soc.* **1989**, *111*, 737.
19. Veldkamp, A.; Frenking, G. *J. Am. Chem. Soc.* **1994**, *116*, 4937.
20. Lohray, B.B.; Bhushan, V. *Tetrahedron Lett.* **1992**, *33*, 5113.
21. Kolb, H.C.; Andersson, P.G.; Bennani, Y.L.; Crispino, G.A.; Jeong, K-S.; Kwong, H-L.; Sharpless K.B. *J. Am. Chem. Soc.* **1993**, *115*, 12226.
22. (a) Corey, E.J.; Noe, M.C. *J. Am. Chem. Soc.* **1993**, *115*, 12579. (b) Corey, E.J.; Noe, M.C. Sarshar, S. *Tetrahedron Lett.* **1994**, *35*, 2861. (c) Corey, E.J.; Noe, M.C.; Grogan, M.J. *Tetrahedron Lett.* **1994**, *35*, 6427.
23. Becker, H.; Ho, P.T.; Kolb, H.C.; Loren, S.; Norrby, P-O.; Sharpless, K.B. *Tetrahedron Lett.* **1994**, *35*,
24. Göbel, T.; Sharpless, K.B. *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1329.
25. (a) Norrby, P-O.; Kolb, H.C.; Sharpless, K.B. *Organometallics* **1994**, *13*, 344. (b) Norrby, P-O Kolb, H.C.; Sharpless, K.B. *J. Am. Chem. Soc.* **1994**, *116*, 8470.
26. Kolb, H.C.; Andersson, P.G.; Sharpless, K.B. *J. Am. Chem. Soc.* **1994**, *116*, 1278.

Q.547
Il6s
1994/95.2

ORGANIC SEMINAR ABSTRACTS

1994- 1995, SEMESTER II

University of Illinois

Serials Department (2)
220 Library
MC-522

Department of Chemistry
Box 68 Roger Adams Laboratory
600 S. Mathews
Urbana, Illinois 61801-3731

June, 1995

Copyright © by The Board of Trustees of the University of Illinois

SEMINAR TOPICS

Semester II, Spring 1995

Page

Silicon as a Tempporary Connection in Organic Synthesis........................ 1
Vito Guagnano

Recent Efforts Toward α-Methylene-γ-Butyrolactones............................. 9
Nathan W. Stehle

Design and Study of Compounds Related to the Enediyne Antibiotic, Neocarzinostatin..17
Perry S. Corbin

Synthesis and Asymmetric Reactivity of Bridged *Ansa*-Bis(Indenyl)-Based Chrial Titanocenes..25
Haibo Jiang

***NOTICE:* Return or renew all Library Materials! The *Minimum* Fee for each Lost Book is $50.00.**

The person charging this material is responsible for its return to the library from which it was withdrawn on or before the **Latest Date** stamped below.

**Theft, mutilation, and underlining of books are reasons for disciplinary action and may result in dismissal from the University.
To renew call Telephone Center, 333-8400**

UNIVERSITY OF ILLINOIS LIBRARY AT URBANA-CHAMPAIGN

CHEMISTRY LIBRARY

AUG 08 1995

AUG 11 1995

SILICON AS A TEMPORARY CONNECTION IN ORGANIC SYNTHESIS

Reported by Vito Guagnano February 23, 1995

INTRODUCTION

The temporary silicon connection method utilizes a removable silicon atom to link two reaction partners which react intramolecularly to form C-X (X = C, O, H) bonds in a regio- and stereoselective fashion. The initial interest in the use of silicon as a temporary tether for the development of new synthetic strategies was concomitant with the discovery of a remarkable chemical transformation known as the Tamao oxidation.[1] This reaction allows for the oxidative cleavage of the Si-C bond to afford alcohols in a variety of organosilicon compounds. Moreover, the oxidation proceeds with retention of configuration, where applicable. Tamao's procedure[1] and Fleming's use[2] of the phenyldimethylsilyl group as a masked hydroxyl have contributed to rendering the silicon connection method particularly attractive. Although initially applied to free radical intramolecular reactions, this method has been extended to several other chemical transformations in the past few years. This review highlights a selection of valuable applications using the temporary silicon tether methodology in organic synthesis.

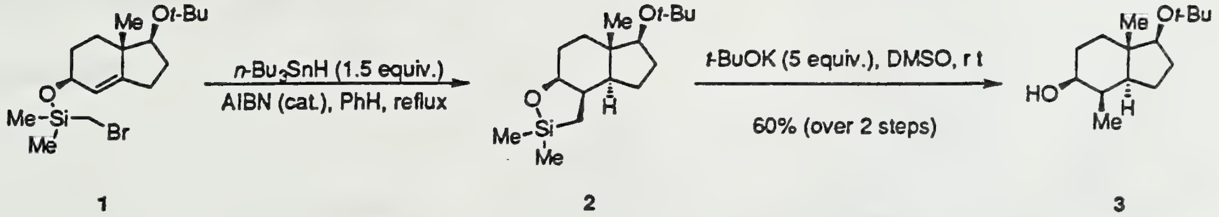
INTRAMOLECULAR FREE RADICAL CYCLIZATIONS

Silicon-tethered Unsaturated Ethers As Radical Acceptors

In the wake of Wilt's mechanistic investigations concerning the cyclization of 2-sila-5-hexen-1-yl radicals,[3] the use of silicon as a temporary connection became recognized as a strategy for the regio- and stereoselective formation of carbon-carbon bonds in free radical processes. In the early 1980s, Nishiyama et al.[4] reported a new approach to the synthesis of 1,3-diols based on the tin hydride mediated cyclization of radicals generated from the corresponding (bromomethyl)-dimethylsilyl allyl ethers, followed by a Tamao oxidation. Unfortunately, the acyclic systems investigated by Nishiyama showed only marginal regio- and stereoselectivity, which appeared to be related to the position of the substituents on the allylic double bond. The application of this methodology to the functionalization of polycyclic allylic alcohols has provided more interesting results,[5] such as Stork's stereoselective introduction of a methyl group in the bicyclic system **1** (Scheme I).[5a] Stereoelectronic effects dictated the regio- (hydroxyl vicinal) and stereochemical (*cis* to the original hydroxyl) outcome in the radical cyclization of silyl ether **1**. Furthermore, due to the shape of the newly formed bicyclic system, the radical formed after initial closure was trapped with tin hydride on the α side of the molecule. Treatment of the intermediate siloxane **2** with potassium *t*-butoxide in dimethyl sulfoxide afforded the methyl substituted alcohol **3** as a single diastereomer.

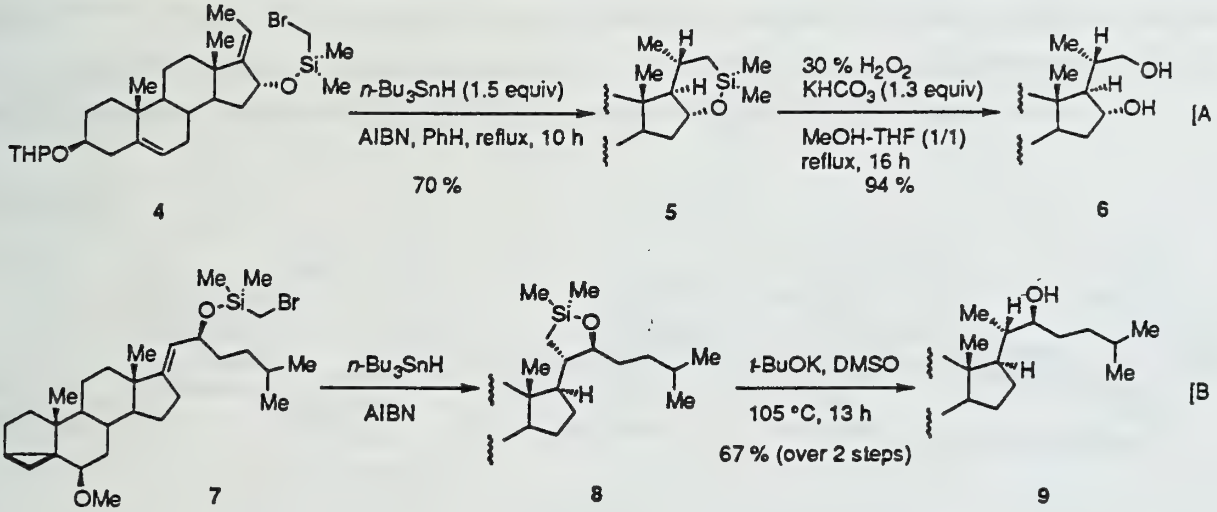
Copyright © 1995 by Vito Guagnano

Scheme I



The method for methylation and hydroxymethylation of allylic alcohols, independently developed by both Nishiyama[4] and Stork,[5a] has been applied to the stereoselective construction of steroid side chains.[6] Representative examples are found in Scheme II. Depending on the stereoelectronic enviroment in which the allyl hydroxy system is placed, either 6-endo[7] (Scheme II, seq. A)[6a] or 5-exo[7] ring closure (Scheme II, seq. B)[6c] can be selectively achieved in the intramolecular cyclization step leading to cyclic siloxanes **5** and **8**, respectively. The stereochemistry of the newly formed stereogenic center in **5** and **8** is determined by the configuration of the carbon bearing the initial allylic hydroxy group.

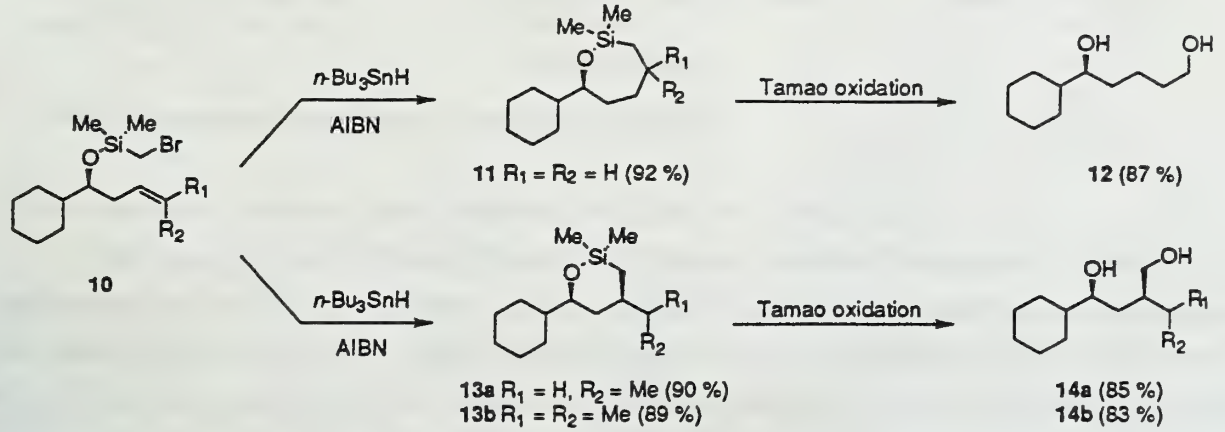
Scheme II



Koreeda has also explored the intramolecular radical cyclization of 2-sila-3-oxa-6-heptenyl radicals, generated from the corresponding (bromomethyl)dimethylsilyl homoallylic ethers, **10**, disclosing a highly stereoselective approach to branched chain 1,4-diols (Scheme III).[8] As previously observed by Nishiyama,[4] the presence of substituents on the distal olefinic carbon influences the regioselectivity of the ring closure. While unsubstituted olefins undergo 7-endo[7] mode cyclization, mono and disubstituted olefins regioselectively cyclize in a 6-exo[7] fashion to produce exclusively six-membered *cis*-siloxanes. Siloxanes **11** and **13a-b** afforded, upon

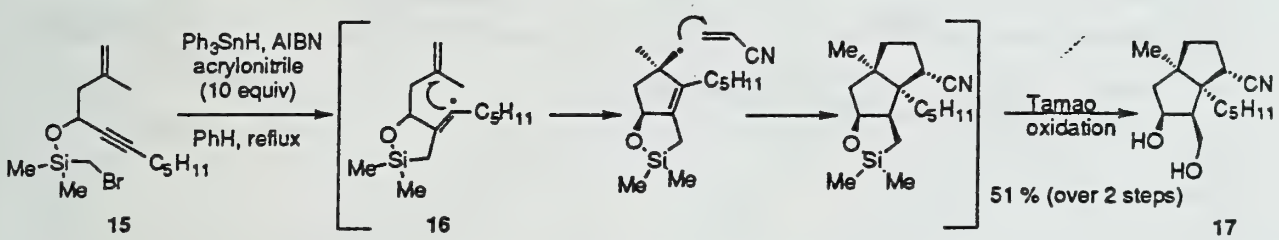
treatment with hydrogen peroxide and potassium bicarbonate in refluxing THF / MeOH, 1,5-diol **12** and 1,4-diols **14a-b**, respectively.

Scheme III



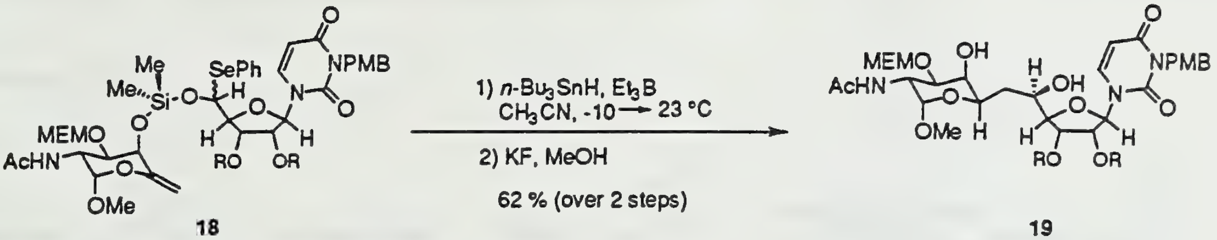
In related work, Malacria has investigated silicon tethered radical cyclizations of alkynes employing propargylic silyl ethers (Scheme IV).[9] In the presence of acrylonitrile, a tandem radical reaction occurs which results in the stereoselective construction of the functionalized diquinane system **17**.[9b,d] The highly diastereoselective formation of racemic **17** is rationalized on the basis of 1,3-asymmetric induction in the cyclization of vinyl radical **16** (presumably through a chair-like transition structure) which is responsible for the creation of all additional stereocenters.

Scheme IV



Lastly, it is worth mentioning the use of silaketals as tethers in intramolecular radical cyclizations.[10] An interesting synthetic application has been reported by Myers and is shown in Scheme V.[10a] The tin hydride promoted radical cyclization of silaketal **18** and hydrolytic removal of the silicon connector are the key steps in the synthesis of the tunicaminyluracil derivative **19**, the undecose core of the tunicamycin antibiotics.

Scheme V

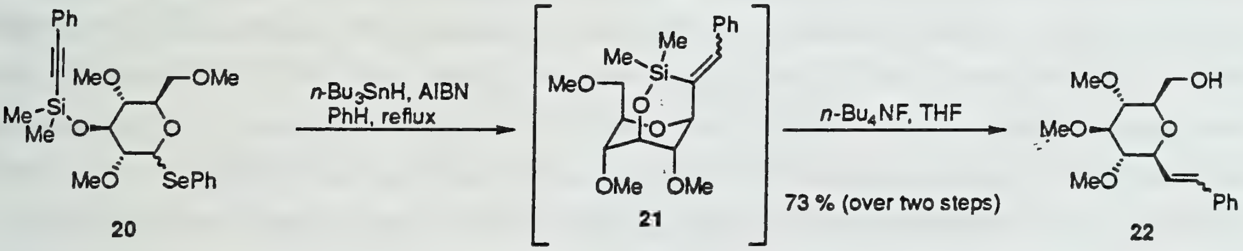


MEM = methoxyethoxymethyl, PMB = *p*-methoxybenzyl, R = TBDMS

Silicon-tethered Alkenes and Alkynes as Radical Acceptors

The use of silicon tethered alkenes and alkynes as radical acceptors in intramolecular cyclizations has provided noteworthy results. In particular, Stork has established a milestone in this field by devising a new method for the stereoselective synthesis of C-glycosides (Scheme VI).[11] The phenylethynyl group, tethered via a silicon atom to a selected hydroxy group of a carbohydrate derivative, is the radical acceptor in the tin hydride mediated cyclization. The reaction results, after removal of the silicon connector, in the stereospecific introduction (*cis* to the tethered hydroxyl) of a styryl group which can be converted to a formyl or carbinol functionality. The most attractive feature of this methodology consists in its generality: either α or β C-glycosides can be selectively obtained in both the pyranose and furanose series.

Scheme VI



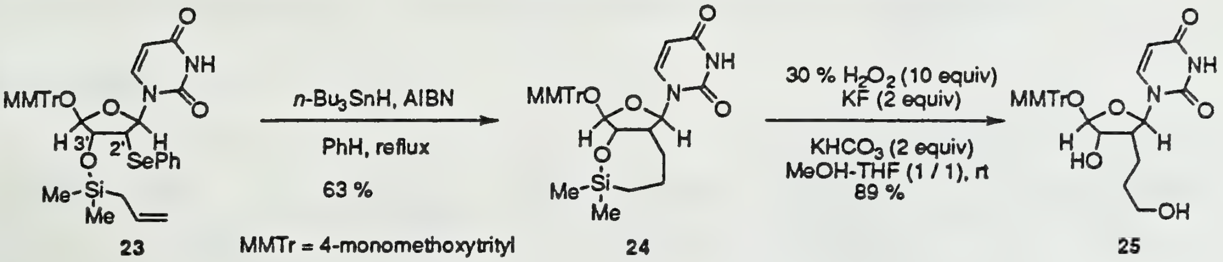
(E / Z > 20 / 1)

Beau and coworkers have modified Stork's method by using samarium iodide as the promoter of the radical cyclization and have applied this protocol to the synthesis of methyl α-C-isomaltoside.[12]

The power of intramolecular free-radical cyclizations has been used by Chattopadhyaya[13] for the stereoselective synthesis of C-branched nucleosides, a class of compounds that exhibit antibacterial and antitumor activities. As shown in Scheme VII, the radical generated at the 2' position of nucleoside derivative 23 was trapped in a 7-endo fashion by the allylsiloxane group. The configuration at the 1' and 3' centers was responsible for the stereochemical outcome of the

cyclization, leading to the *cis*-fused seven-membered bicyclic siloxane **24**. Tamao oxidation afforded the 1,5-diol **25** in good overall yield (56 %).[13a]

Scheme VII



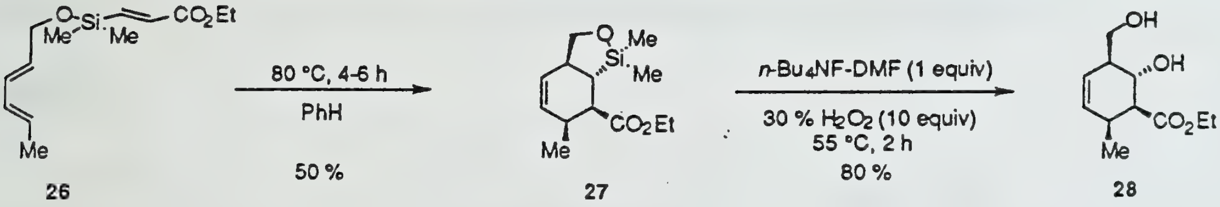
Silicon-tethered Radical Cations In Electrochemically Initiated Cyclizations

Recently, Moeller has employed a temporary silicon connection in radical cyclizations, where the initial radical species is oxidatively generated in an electrochemical reaction.[14] This work provides the first example of an intramolecular anodic olefin coupling reaction involving the use of a tethered vinylsilane in the construction of quaternary carbons. The scope and limitation of this methodology are currently under investigation.

CYCLOADDITIONS

The temporary silicon connection method has proven to be a powerful tool for regio- and stereocontrol in cycloadditions, including the Diels-Alder reaction.[15] In this context, the synthetic utility is well illustrated by the synthesis of the highly substituted cyclohexene **28** reported by Stork (Scheme VIII).[15d] The thermally induced cyclization of the (*E*)-acrylate **26** afforded diastereomerically pure cyclic siloxane **27**. Compound **27**, with four contiguous stereogenic centers, is the product of an endo (carboethoxy) approach of the dienophile to the diene in the carbon-carbon bond forming event. Oxidative cleavage of the silicon tether gave the 1,3-diol **28** in 40 % overall yield.

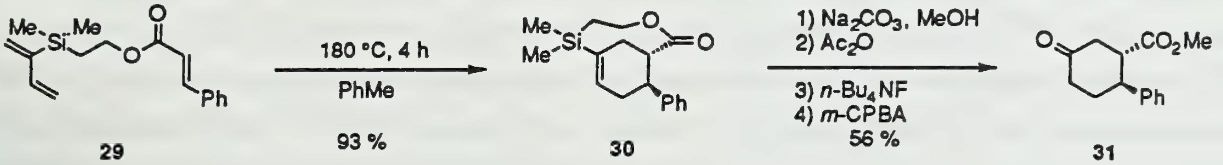
Scheme VIII



Shea has extended the use of a temporary silicon tether to type 2 intramolecular Diels-Alder reactions, in which the dienophile is tethered to one of the internal sp^2 carbons of the diene.[15a,b]

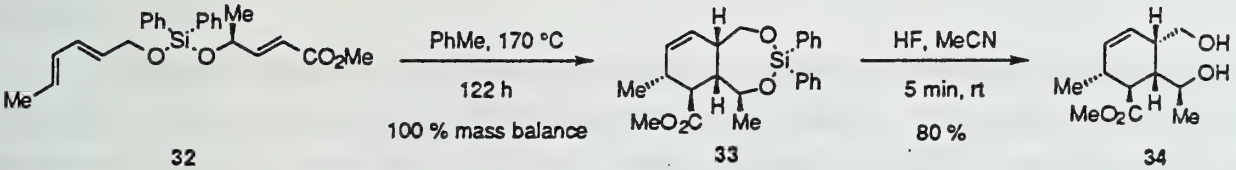
The geometrical constraints imposed by the tether reverse the regiochemistry typically observed in Diels-Alder reactions. Shea refers to this phenomenon as *pericyclic umpolung*. In Scheme IX, the thermal cycloaddition of the (*E*)-cinnamic ester **29** exclusively afforded the bridged cycloadduct **30** in 93 % yield. A four-step process was required to convert compound **30** into the *trans*-disubstituted cyclohexanone **31**, which was obtained in good overall yield (56 %).[15b]

Scheme IX



Craig and Reader have utilized a diphenyl silaketal unit to connect the diene and dienophile components in the [4 + 2] cycloaddition in Scheme X.[15e] The cyclization of silaketal **32** proceeded with complete regioselectivity and exhibited exclusive diastereo- and enantioselectivity, which was the result of effective relative asymmetric induction. Upon treatment with hydrofluoric acid in acetonitrile, compound **33** afforded diol **34** in good yield (80 %).

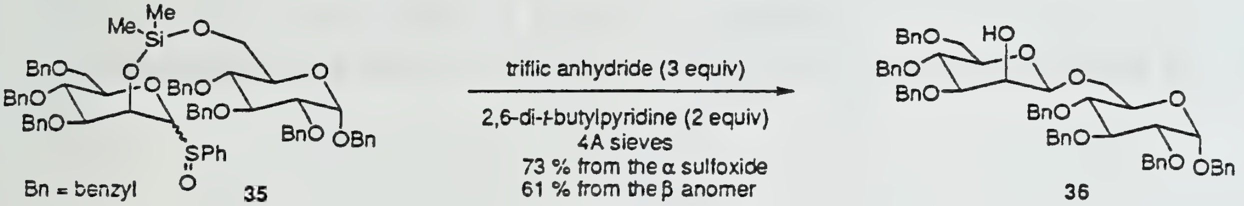
Scheme X



SYNTHESIS OF DISACCHARIDES

Although silaketals have been successfully used as tethering units in intramolecular free radical cyclizations and [4 + 2] cycloadditions, their synthetic utility is further enhanced by Stork's approach to the synthesis of disaccharides.[16] Scheme XI describes the formation of 6-*O*-glucosyl-β-D-mannopyranoside **36**, arising from the exposure of silaketal **35** to triflic anhydride and 2,6-di-*t*-butylpyridine. The net result of the reaction is an intramolecular glycosylation.

Scheme X

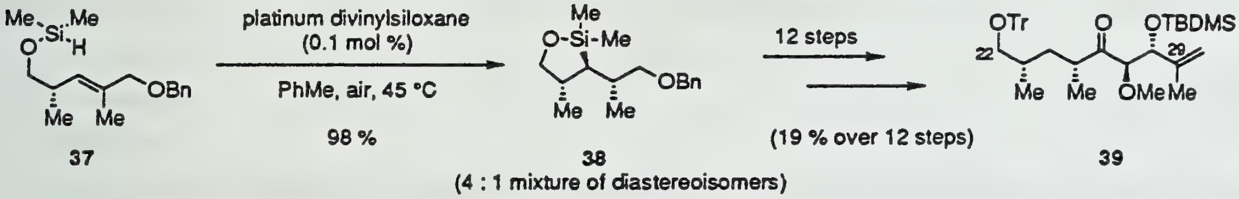


HYDROSILYLATIONS

Intramolecular hydrosilylations[17] can be considered to be an application of the temporary silicon connection method, even though the silicon atom is one of the reaction components. Within this realm, asymmetric catalytic hydrosilylations of olefins have been reported by Bosnich.[17b] Unfortunately, the applicability of this methodology to the enantioselective synthesis of alcohols and diols is severely limited since satisfactory levels of asymmetric induction are achieved only in the case of aryl substituted olefins.

More interesting is Hoveyda's use of hydrosilylation (Scheme XII) in the stereoselective synthesis of the C22-C29 segment (39) of the immunosuppressant rapamycin.[17e] Cyclic siloxane **38**, a key intermediate of this synthesis, contains three stereogenic centers that are formed with 4 : 1 diastereoselectivity. This compound plays a crucial role by preserving and communicating asymmetry during the construction of the aliphatic chain.

Scheme XII



CONCLUSION

The silicon atom has proven to be a remarkable connector in a number of organic reactions, such as free radical cyclizations, cycloadditions and hydrosilylations. This strategy allows for the synthesis of highly functionalized cyclic adducts which can be converted to useful products by the efficient removal of the silicon linker. These compounds are formed with high levels of regio- and stereoselectivity, which otherwise could not be achieved from the corresponding intermolecular processes.

REFERENCES

(1) (a) Tamao, K.; Ishida, N.; Tanaka, T.; Kumada, M. *Organometallics* **1983**, *2*, 1694-1696. (b) Tamao, K.; Ishida, N. *J. Organomet. Chem.* **1984**, *269*, C37-C39. (c) Tamao, K.; Kumada, M. *Tetrahedron Lett.* **1984**, *25*, 321-324.

(2) Fleming, I.; Sanderson, P. E. J. *Tetrahedron Lett.* **1987**, *28*, 4229-4232.

(3) Wilt, J. W. *J. Am. Chem. Soc.* **1981**, *103*, 5251-5253.

(4) Nishiyama, H.; Kitajima, T.; Matsumoto, M.; Itoh, K. *J. Org. Chem.* **1984**, *49*, 2298-2300.

(5) (a) Stork, G.; Kahn, M. *J. Am. Chem. Soc.* **1985,** *107*, 500-501. (b) Stork, G.; Sofia, M. J. *J. Am. Chem. Soc.* **1986,** *108*, 6826-6828. (c) Stork, G.; Mah, R. *Tetrahedron Lett.* **1989,** *30*, 3609-3612. (d) Tamao, K.; Maeda, K.; Yamaguchi, T.; Ito, Y. *J. Am. Chem. Soc.* **1989,** *111*, 4984-4985.

(6) (a) Koreeda, M.; George, I. A. *J. Am. Chem Soc.* **1986,** *108*, 8098-8100. (b) Koreeda, M.; George, I. A. *Chem. Lett.* **1990,** 83-86. (c) Kurek-Tyrlik, A., Wicha, J., Zarecki, A. *J. Org. Chem.* **1990,** *55*, 3484-3492.

(7) Baldwin, J. E. *J. Chem. Soc., Chem. Commun.* **1976,** 734-736.

(8) Koreeda, M.; Haman, L. G. *J. Am. Chem. Soc.* **1990,** *112,* 8175-8177.

(9) (a) Journet, M.; Magnol, E.; Agnel, G.; Malacria, M. *Tetrahedron Lett.* **1990,** *31*, 4445. (b) Journet, M.; Smadja, W.; Malacria, M. *Synlett* **1990,** 320-321. (c) Hutchinson, J. H.; Daynard, T. S.; Gillard, J. W. *Tetrahedron Lett.* **1991,** *32*, 573-576. (d) Journet, M.; Malacria, M. *J. Org. Chem.* **1992,** *57*, 3085-3093.

(10) (a) Myers, A. G.; Gin, D. Y.; Widdowson, K. L. *J. Am. Chem. Soc.* **1991,** *113*, 9661-9663. (b) Myers, A. G.; Gin, D. Y.; Rogers, D. H. *J. Am. Chem. Soc.* **1993,** *115*, 2036-2038.

(11) Stork, G.; Suh, H. S.; Kim, G. *J. Am. Chem. Soc.* **1991,** *113*, 7054-7056.

(12) Mazéas, D.; Skrydstrup, T.; Doumeix, O.; Beau, J. *Angew. Chem. Int. Ed. Engl.* **1994,** *33*, 1383-1386.

(13) (a) Xi, Z.; Agback, P.; Plavec, J.; Sandström, A. J.; Chattopadhyaya, J. *Tetrahedron* **1992,** *48*, 349-370. (b) Xi, Z.; Rong, J.; Chattopadhyaya, J. *Tetrahedron* **1994,** *50*, 5255-5272.

(14) (a) Moeller, K. D.; Hudson, C. M.; Tinao-Wooldridge, L. V. *J. Org. Chem.* **1993,** *58*, 3478-3479. (b) Hudson, C. M.; Moeller, K. D. *J. Am. Chem. Soc.* **1994,** *116*, 3347-3356.

(15) (a) Shea, K. J.; Zandi, K. S.; Staab, A. J.; Carr, R. *Tetrahedron Lett.* **1990,** *31*, 5885-5888. (b) Shea, K. J.; Staab, A. J.; Zandi, K. S. *Tetrahedron Lett.* **1991,** *32*, 2715-2718. (c) Gillard, J. W.; Fortin, R.; Grimm, E. L.; Maillard, M.; Tjepkema, M.; Bernstein, M. A.; Glaser, R. *Tetrahedron Lett.* **1991,** *32*, 1145-1148. (d) Stork, G.; Chan, T. Y.; Breault, G. A. *J. Am. Chem. Soc.* **1992,** *114*, 7578-7579. (e) Craig, D.; Reader, J. C. *Tetrahedron Lett.* **1992,** *33*, 4073-4076. (f) Sieburth, S. M.; Fensterbank, L. *J. Org. Chem.* **1992,** *57*, 5279-5281. (g) Rumbo, A.; Castedo, L.; Mouriño, A.; Mascareñas, J. L. *J. Org. Chem.* **1993,** *58*, 5585-5586.

(16) Stork, G.; Kim, G. *J. Am. Chem. Soc.* **1992,** *114*, 1087-1088.

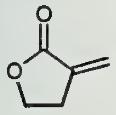
(17) (a) Tamao, K.; Nakagawa, Y.; Ito, Y. *J. Org. Chem.* **1990,** *55*, 3438-3439. (b) Bergens, S. H.; Noheda, P.; Whelan, J.; Bosnich, B. *J. Am. Chem. Soc.* **1992,** *114*, 2121-2128. (c) Bergens, S. H.; Noheda, P.; Whelan, J.; Bosnich, B. *Ibid.* **1992,** *114*, 2128-2135. (d) Denmark, S. E.; Forbes, D. C. *Tetrahedron Lett.* **1992,** *33*, 5037-5040. (e) Hale, M. R.; Hoveyda, A. H. *J. Org. Chem.* **1992,** *57*, 1643-1645.

RECENT EFFORTS TOWARD α-METHYLENE-γ-BUTYROLACTONES

Reported by Nathan W. Stehle February 27, 1995

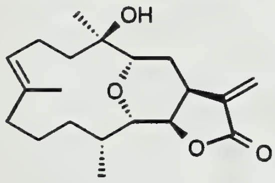
INTRODUCTION

The occurrence of α-methylene-γ-butyrolactones in natural products has been widely reported.[1] Numerous substances with this particular structural feature show marked bioactivity, and many more bioactive compounds are certain to be discovered.[1] The development of new viable pathways to α-methylene-γ-butyrolactones is a constant feature in the literature. The chemical diversity of these compounds ranges from tulipalin A (**1**), the simplest α-methylene-γ-butyrolactone, to the cembranoid diterpene eunicin (**2**), which is one of the most complex molecules containing an α-methylene-γ-butyrolactone unit.



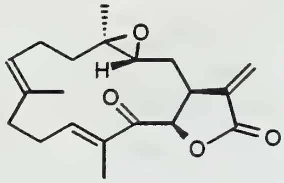
tulipalin A

1



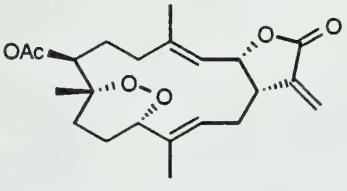
eunicin

2



peunicin

3



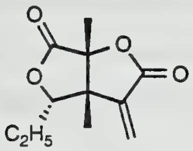
denticulatolide

4

BIOACTIVITY

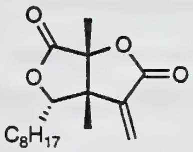
Several α-methylene-γ-butyrolactones exhibit beneficial bioactivity, but the high toxicity of most limits their widespread clinical use.[1,2] Peunicin (**3**), a cembranolide from *Eunicea mammosa-succinea*, exhibited anticiliary action and is cytotoxic.[3] Some cembranoid diterpenes containing α-methylene-γ-butyrolactones such as denticulatolide (**4**) are ichthyotoxic.[4]

Non-cembranoid diterpenoids with the α-methylene-γ-butyrolactone unit also exhibit substantial activity. Ethisolide (**5**),[5] isoavenaciolide (**6**),[5] and avenaciolide (**7**)[6] are



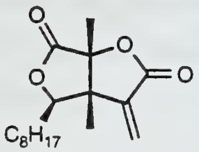
ethisolide

5



isoavenaciolide

6



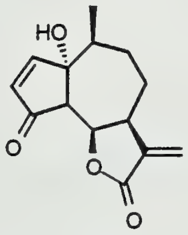
avenociolide

7

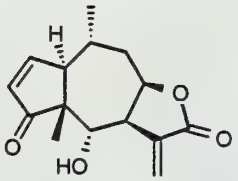
Copyright © 1995 by Nathan W. Stehle

all secondary metabolites from the fermentation of *Aspergillus* and *Penicillium* species.[7] Of the three, avenaciolide has the widest range of biological properties including the inhibition of fungal spore germination,[6] antibacterial action,[6] and inhibition of glutamate transport in rat liver mitochondria.[8]

One effect that α-methylene-γ-butyrolactones have on humans is allergic contact dermatitis (ACD). Parthenin (**8**), present in the herb *Parthenium hysterophoros*, is the major dermatological allergen that occurs in India and neighboring countries. A structurally related compound helenalin (**9**) is a fungicidal and anthelminthic substance found in the common sunflower *Helianthus annuus L.*[9] The toxic effects usually arise from a 1,4-conjugate addition of an L-cysteine thiol to the α-methylene-γ-butyrolactone.



parthenin
8



heleanalin
9

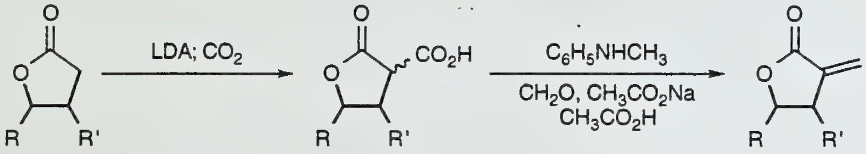
SYNTHESIS

This review will generally focus on methodologies developed within the past six years to synthesize compounds with the α-methylene-γ-butyrolactones substructure.[1]

Methylenation of Lactones

Many syntheses simply involve introduction of a methylene group on an existing lactone ring. Often methylenations involve α-amino or α-hydroxymethylenation followed by an elimination or decarboxylation sequence.[10] In attempts to avoid ring opening and double bond isomerization, Greene and co-workers developed a mild method for methylenations via decarboxylation (Scheme I).[10] Mild conditions are achieved by the use

Scheme I



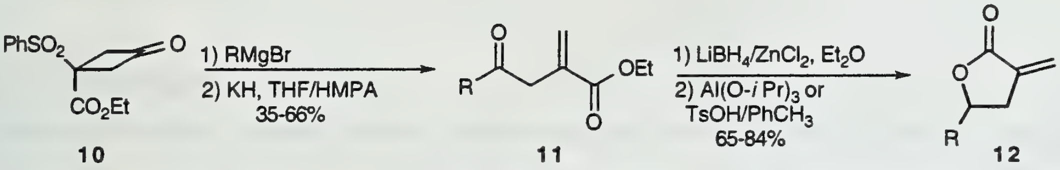
R=H; n-$C_{10}H_{21}$; Ph
R'=Ph; p-C_6H_4F; n-C_6H_{13}; H; n-$C_{13}H_{27}$

of buffer to stabilize the pH of the system affording the desired α-methylene-γ-butyrolactone, and may be a fairly general solution to methylenations.

Novel Methodologies

Takeda and co-workers have developed a route to substituted α-methylene-γ-butyrolactones **12** starting from 3-ethoxycarbonyl-3-(phenylsulfonyl)-cyclobutanones **10**.[11] The construction of the lactone ring with the α-methylene unit in place, leading to a concise, efficient method for installation of both sought functionalities (Scheme II). The route is notable, because it uses a carbanion to install the γ-substituent. Other procedures involve the attack of aldehydes with enolates bearing an ester functionality or its equivalent.[1,11]

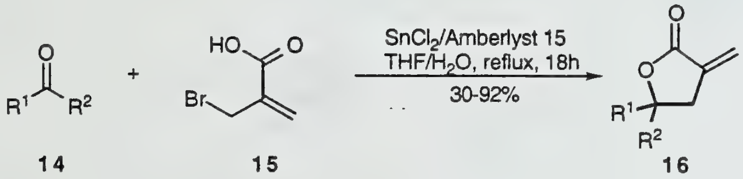
Scheme II



R=CH$_3$; CH$_3$(CH$_2$)$_3$; Ph; 5-methyl-2-furyl; 2-thienyl

Stampf accessed γ-substituted α-methylene-γ-butyrolactones, **16**, via a Reformatzky-type reaction using SnCl$_2$, Amberlyst-15 resin and aldehyde **14** (Scheme III).[12] The process permits later modifications when a brominated alkyl is present (R=Br(CH$_2$)$_{10}$). Similarly, Wu and co-workers obtained γ,γ-disubstituted α-methylene-γ-butyrolactones in good to excellent yields using a procedure with powdered tin.[16] Functionalities such as nitro, hydroxy and halogen[13] survive the mild conditions, permitting possible application of this method of α-methylene-γ-butyrolactone formation.

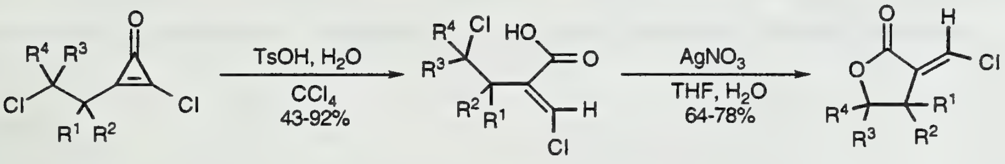
Scheme III



R^1=Ph; PhCH$_2$; Et; *n*-C$_6$H$_{13}$; Ph=CH$_2$; Br(CH$_2$)$_{10}$
R^2=H; R^{1+2}= -(CH$_2$)$_5$-

Mayr used cylopropenones as precursors for the synthesis of several substituted compounds (Scheme IV).[14] The α-methylene-γ-butyrolactone, **19**, skeleton was synthesized in two steps from the cyclopropenone, **17**, and the reductive removal of the chloro substituent gave the desired lactone. Only alkyl substituents have been introduced in but other functional groups need to be tested to expand the utility of this methodology.

Scheme IV



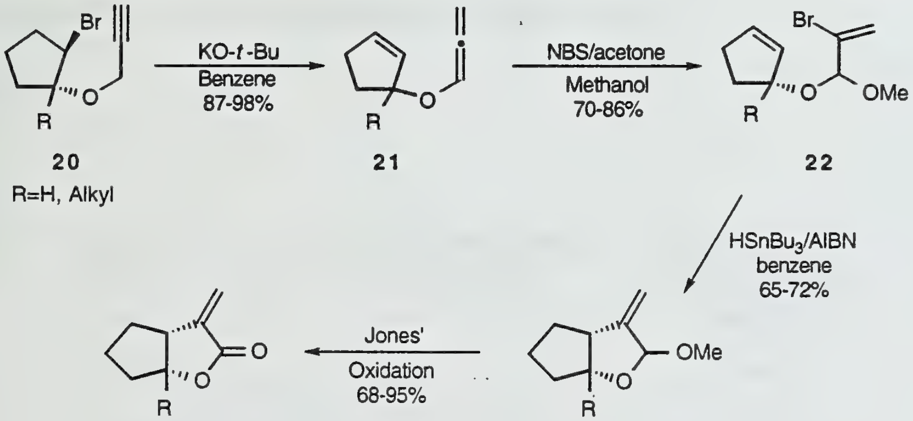
17 **18** **19**

R^1,R^2,R^3,R^4=Alkyl

Radical Cyclizations

Many cyclization methods involve reactive radical intermediates since the known reactivity patterns allow controlled annulations. Given the wide array of radical pathways, it is surprising there are not more syntheses for α-methylene-γ-butyrolactones exploiting intramolecular radical ring closure. Radical methodologies afford highly selective *cis* ring closure products but difficulty arises when a *trans* ring fusion is required.

Scheme V



20 **21** **22**

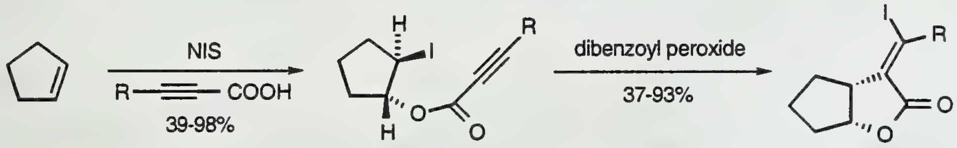
R=H, Alkyl

24 **23**

In conjunction with efforts to form Botryodiplodin derivatives Dulcère developed allenyl ethers, **21**, as a route to α-methylene-γ-butyrolactones (Scheme V). Conversion of allenyl ether **21** to lactone **24** was achieved by formation of the acyclic acetal **22** followed by cyclization and oxidation.[15] The final chromium promoted oxidation proceeds well, but is not industrially useful.

Weavers and co-workers performed a radical addition to a triple bond to construct quickly the iodo α-methylene-γ-butyrolactone **27** (Scheme VI).[16] The best selectivity was achieved when the radical cyclization was initiated with dibenzoyl peroxide, and only the (*E*) isomer was formed. The selectivity of this reaction and the reactivity of iodo compounds may provide modifications of α-methylene-γ-butyrolactones and permit their adaptation to natural product syntheses.

Scheme VI



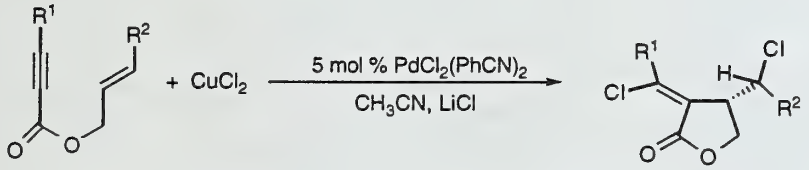
25 **26** **27**

R=H, Me, SiMe$_3$

Cyclization Aided by Transition Metals

Palladium catalysis often used in coupling reactions was also found to be useful in the synthesis of α-methylene-γ-butyrolactones. Pd(II) catalyzed cyclization of 2'-alkenyl-2-alkynoates, **28**, (Scheme VII) in the presence of cuprous chloride and lithium chloride was found to be an efficient route to synthesize α-(chloromethylene)-γ-butyrolactones,[17] with the (*Z*) isomer the major product (69-100% yield, 95-100% *Z*) in the cyclization of 2'-alkenyl-2-propynoates. The reaction proceeds via a π-allyl intermediate mediated by CuCl$_2$

Scheme VII



28 **29**

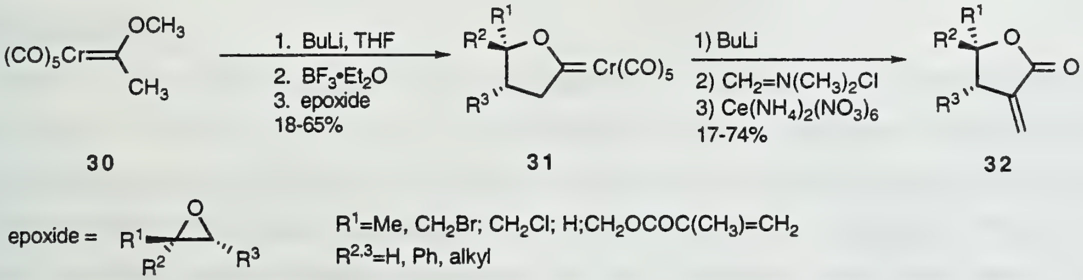
R^1=CH$_3$; *n*-Bu
R^2=H, Ph

and palladium, and not a radical mechanism as previously postulated.[17] The non-radical mechanism was proposed because the use of *tert*-butyl nitroxide failed to trap a radical and ESR

studies failed to detect any radicals corresponding to expected intermediates.[17] The role of the LiCl is unclear, although it was observed to increase the (*Z*)-selectivity of the reaction to provide **29**.

The reaction of chromium carbenes with epoxides was adapted by Papagni as a powerful new tool for asymmetric synthesis of substituted α-methylene-γ-butyrolactones (Scheme VIII).[18] The reaction proceeded via an attack by the carbene-type enolate of **30** at the least substituted end of the epoxide with inversion of the stereochemistry.[18] This inversion caused incompatibility with substrates that do not tolerate a *trans* ring formation, such as cyclopentene oxides. The α-methylene introduced by the treatment of **31** with base followed by addition of Eschenmoser's salt gave the α-methylene-γ-butyrolactones **32**. The predictability of the product and wide array of possible product stereochemistry and availability of substituted epoxides allows the application of this methodology to synthesize numerous α-methylene-γ-butyrolactones.

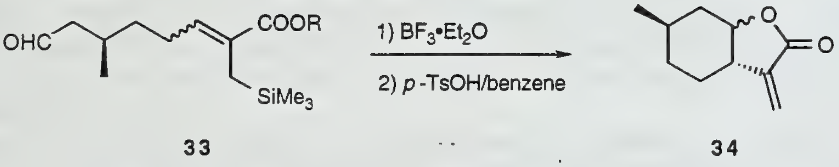
Scheme VIII



Additional Asymmetric Methods

In the Hosomi reaction allyl silanes, **33**, are the precursors constructed for the selective synthesis of *cis* and *trans* methanolides (Scheme IX).[19] When optically active substrates are employed, the six-membered ring formation affords two diastereomers (*Z*) and (*E*), which are then separated and each cyclized to form the *cis* and *trans* *p*-menthanolides **34** respectively as the sole product.[19] The formation of the substrate

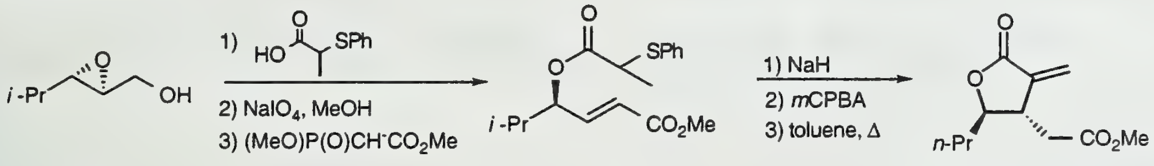
Scheme IX



requires several steps, but all the required functionalities are installed at the time of cyclization. The applicability may be limited to five and six membered ring formation of substituted bicyclic α-methylene-γ-butyrolactones, but there are numerous possibilities for new compounds within those parameters.

Rodriguez and Martín[20] employ optically active epoxides, **35**, selective ring opening with 2-phenylmercapto-propionic acid to give the diol ester which was then oxidatively cleaved and submitted to Horner-Emmons conditions to give **36**. Deprotonation with subsequent 1,4 conjugate addition formed the lactone ring. Formation of the sulfoxide and thermal elimination gave **37**. The asymmetric epoxidation of allylic alcohols affords controlled stereochemistry of substrates.

Scheme X



35 **36** **37**

CONCLUSIONS

New and modified procedures for preparation of α-methylene-γ-butyrolactones will continue to be a focus in the literature.[1,17,18] Methylenations are established methodologies that can be readily performed on lactones.[10] However numerous novel methodologies exist that have the methylene unit in place upon cyclization which avoids the need for additional steps.[11,12,13,14] Common cyclization methodologies using radicals and palladium have also been effectively extended to α-methylene-γ-butyrolactone synthesis, which permit selective cyclizations.[15,16,17] Asymmetry can be introduced utilizing optically active compounds such as optically active epoxides and natural products as starting materials.[18,19,20] With the varied approaches researchers have taken, further growth in cyclization methodologies will aid in the synthesis of new bioactive natural products as well as derivatives are beneficial to people, plants and animals.

REFERENCES

1. (a) Hoffmann, H. M. R.; Rabe, J. *Angew Chem. Int. Ed. Engl.* **1985**, *24*, 94-110. (b) Petragnani, N.; Ferraz, H.M.C.; Silva, G. V. J. *Synthesis*, **1986**, 157-183. (c) Sarma, J. C.; Sharma, R. P. *Heterocycles* , **1986**, *24*, 441-457.
2. Gampe, R.T.; Alam, M.; Weinheimer, A. J.; Martin, G. E.; Matson, J. A.; Willcott, III, R. W.; Inners, R. R.; Hurd, R. E. *J. Am. Chem. Soc.* **1984**, *106*, 1823-1826.
3. Chang, C. Y.; Ciereszko, L. S.; Hossain, M. B.; Van Der Helm, D. *Acta Cryst* . **1980**, B*36*, 731-733.
4. Fukazawa, Y.; Usui, S.; Uchio, Y. *Tetrahedron Lett.* **1986**, *27*, 1825-1828.
5. Aldridge, D.C.; Turner, W.B.; *J. Chem. Soc. Chem. C* **1971**, 2431-2432.
6. Brookes, D.; Tidd, B.K.; Turner, W. B. *J. Chem. Soc.* **1963**, 5385-5391.
7. Burke, S. D.; Pacofsky, G.J.; Psicopio, A.D. *J. Org. Chem.* **1992**, *57* , 2228-2235.
8. Meyer, J.; Vignais, P.M. *Biochim Biophys. Acta.* **1973**, *325*, 379.
9. Grieco, P. In*Stereoselective Synthesis of Natural Products*; Bartmann, W.; Winterfeldt, E.,Ed.; Excerpta Medica Workshop Conferences Hoechst: Amsterdam, **1979**; p. 121.

10. Greene, A. E.; Murta, M. M.; de Azevedo, M. B. M. *Synth. Commun.* **1993**, *23*, 495-503.
11. Fujiwara, T.; Morita, K.; Takeda, T. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 1524-1527.
12. Talaga, P.; Schaeffer, M.; Benezra, C.; Stampf, J. *Synthesis*, **1990**, 530.
13. Zhou, J.; Lu, G.; Wu, S. *Synth. Commun.* **1992**, *22*, 481-487.
14. Musigmann, K.; Mayr, H. *Tetrahedron Lett.* **1990**, *31*, 1261-1264.
15. Dulcère, J.; Mihoubi, M. N.; Rodriguez, J. *J. Org. Chem.* **1993**, *58*, 5709-5716.
16. Weaver, R. T.; Haaima, G. *Tetrahedron Lett.* **1988**, *29*, 1085-1088.
17. Ma, S.; Lu, X. *J. Org. Chem.* **1993**, *58*, 1245-1250.
18. (a) Lattuada, L.; Licandro, E.; Maiorana, S.; Papagni, A. *Gazz. Chim. Ital.* **1993**, *123* , 31-33. (b) Lattuada, L.; Licandro, E.; Maiorana, S.; Molinari, H.; Papagni, A. *Organometallics*, **1991**, *10*, 807-812.
19. Nishitani, K.; Fukuda, K.; Yamakawa, K. *Heterocycles*, **1992**, *33*, 97-100.
20. Rodríguez, C. M.; Martín, V. S. *Tetrahedron Lett* . **1991**, *32*, 2165-2168.

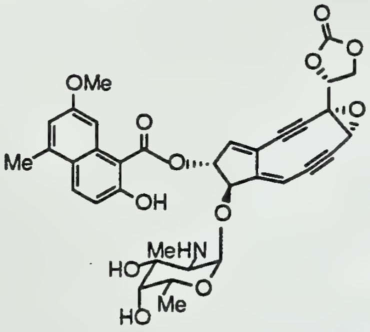
DESIGN AND STUDY OF COMPOUNDS RELATED TO THE ENEDIYNE ANTIBIOTIC, NEOCARZINOSTATIN

Reported by Perry S. Corbin March 2, 1995

INTRODUCTION

Neocarzinostatin (NCS) is a member of a rapidly growing group of antitumor agents known as the enediyne antibiotics.[1] Despite being the first member of the class to be isolated and characterized biologically, interest in NCS did not increase until the discovery of the antibiotic's more well known relatives, calicheamicin γ_1 and esperamicin A_1.[2]

The antibiotic NCS was isolated in 1965 from *Streptomyces carzinostaticus* and was shown to have significant antimicrobial and chemotherapeutic activity—believed to be linked to the DNA cleaving properties of the antibiotic.[3] At the time of its discovery, NCS was believed to be a protein. However, later studies demonstrated that the antibiotic actually exists as a "chromoprotein" complex—with the apoprotein portion serving primarily as a stabilizing unit for the biologically active neocarzinostatin chromophore component **1** (Figure 1).[4]



1

Figure 1: Neocarzinostatin chromophore (NCS-chromophore)

The structure of **1**, as well as its complete stereochemistry, was reported in the 1980's and has recently been verified by crystallographic analysis of the holoprotein.[5,6] Since the initial report of the structure of the chromophore and its DNA cleaving properties, considerable research has been carried out in attempts to elucidate the chromophore's mechanism of action and to synthesize molecules which are structurally related to **1**. The synthesis of these molecules, as well as the structural and mechanistic features of **1** which have guided their synthesis, will be examined in this abstract.

MECHANISM OF ACTION OF NCS-CHROMOPHORE

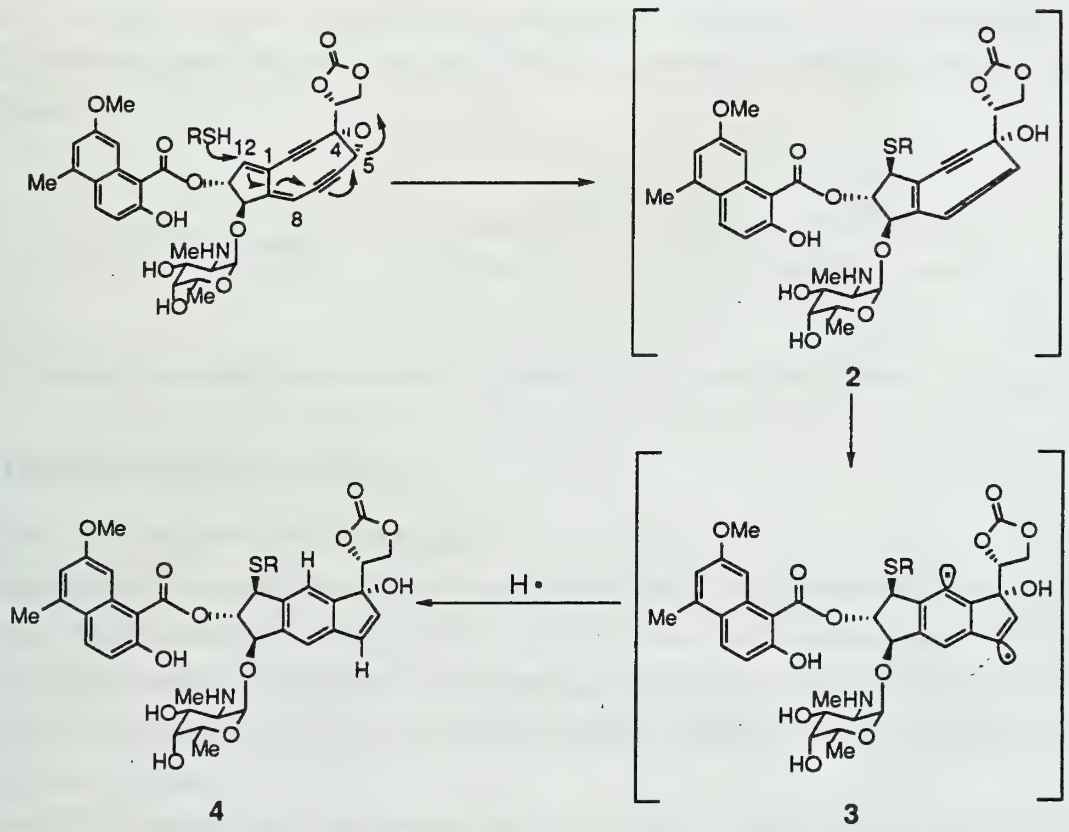
Prior to the elucidation of the structure of **1**, DNA cleavage by the antibiotic was shown to be primarily an oxygen and thiol dependent process.[7] These and other observations, including a critical study

Copyright © 1995 by Perry S. Corbin

by Goldberg in which tritium was incorporated into the chromophore upon reaction of **1** with 2-mercaptoethanol and [5′-^{3}H] thymidine labeled oligonucleotide, poly (dA, dT), suggested that a radical species was responsible for the antibiotic's biological activity.[8]

Furthermore, in 1983, Goldberg and co-workers reported that **1** reacts with methylthioglycolate to produce a 1:1 adduct (which has incorporated two new hydrogens) as the major product.[9] Subsequent work on this reaction by Myers led to the identification of the 1:1 adduct **4** and to a mechanistic proposal (Scheme I) which not only explains the formation of **4**, but also explains the chromophore's ability to cleave DNA by hydrogen abstraction.[10]

Scheme I



The primary step of activation includes an opening of the epoxide group by stereospecific, nucleophilic attack of thiol (glutathione, *in vivo*) at C-12 of the chromophore—believed to be assisted by the nitrogen of the fucosamine.[11] Cycloaromatization of cumulene **2**, resembling the Bergman cyclization process of other enediyne antibiotics,[1,12] leads to diyl **3** which, upon hydrogen abstraction, yields adduct **4**. It is diyl **3** that is believed to generate carbon radicals at C-5′, C-4′, and C-1′ of deoxyribose in DNA. The resultant radicals then create single or double stranded lesions in the DNA via an oxygen dependent process. Deuterium labeling studies, as well as recent studies in which the observable species **2** is shown to be an intermediate in DNA cleavage,[13] have given support for this mechanism.

GENERAL DESIGN OF ANALOGS

Although analogs of **1** have been studied for a variety of reasons, ranging from synthetic and mechanistic curiosity to new-drug development, the design of the molecules has depended critically upon structural and mechanistic features discussed in the previous section. In general, two different types of analogs have been synthesized, macrocyclic and non-macrocyclic.

Most non-macrocyclic analogs have the key features shown in Figure 2a, a cross conjugated dienediyne system with a propargylic electron acceptor. Similarly, functional macrocyclic analogs incorporate the dienediyne and propargyl acceptor of **1**, as well as a macrocyclic ring (Figure 2b). In addition to the functional macrocylic analogs, those which incorporate structural features necessary for a reaction cascade similar to Scheme I, a variety of non-functional 9-membered ring analogs have been synthesized. The primary goal of synthesizing analogs of this type is to develop facile methods of assembling the 9-membered ring in **1** and other natural products. Examples of each of these types are discussed below.

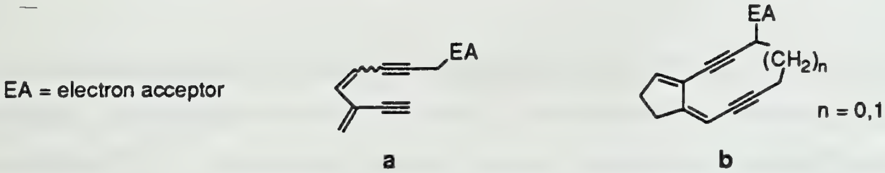


Figure 2: a) General structure of nonmacrocyclic analogs b) General structure of macrocyclic analogs

NON-MACROCYCLIC ANALOGS

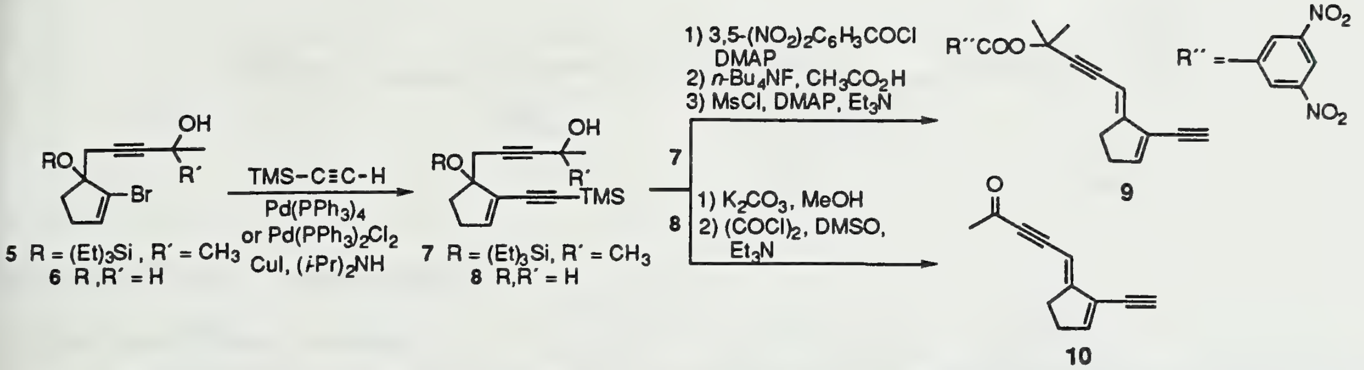
Synthesis of Non-Macrocyclic Analogs

Non-macrocyclic analogs have been synthesized primarily as mechanistic probes. However, the study of these molecules has also led to synthetic strategies which have been used in the design of more complex macrocyclic analogs. Specifically, these analogs demonstrate the flexibility and usefulness of the vinyl halide-acetylene coupling reaction (palladium/copper catalyzed) in the synthesis of the enediyne antibiotics and their mimics.

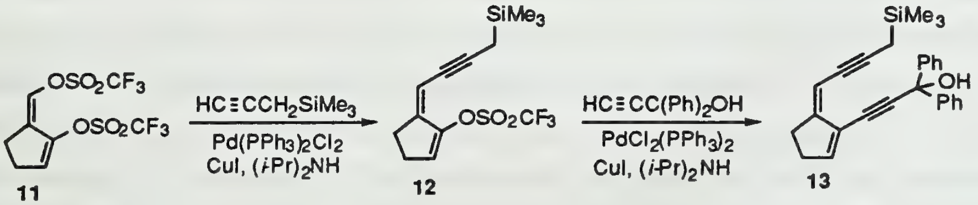
Structurally, Hirama's analog **9** and Wender's analog **10** are similar in their incorporation of an *E*-exocyclic double bond.[14,15] The synthesis of both of these molecules (Scheme II) proceeded through similar intermediates **5** and **6**, synthesized from 2-bromo-2-cyclopentenone, and incorporated a regio- and stereoselective dehydration in the last step of the scheme.

Alternatively, the syntheses of analogs with a *Z*-exocylic double bond have been designed for potential use in the synthesis of non-macrocyclic, as well as macrocyclic analogs.[16,17] An example of a non-macrocyclic analog incorporating a *Z*-exocyclic double bond is given in Scheme III. Compound **13** was synthesized via selective acetylenic coupling to *Z*-bistriflate **11**, available stereoselectively from 2-formylcyclopentanone, followed by coupling of the monotriflate **12** with diphenyl propargyl alcohol.

Scheme II

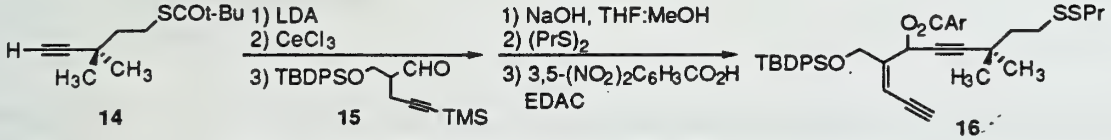


Scheme III



In addition to the aforementioned analogs which incorporate a cyclopentenyl core, acyclic analogs of **1** have been synthesized. An elegant example of such a compound was recently reported by Myers.[18] Analog **16** was synthesized in a convergent manner through a cerium-mediated acetylide addition of compound **14** to **15** (Scheme IV). A cleverly incorporated disulfide unit in **16** can be cleaved, reminiscent of the activation of calicheamicin,[1,2] to reveal an internal nucleophile capable of activating the molecule for cycloaromatization.

Scheme IV

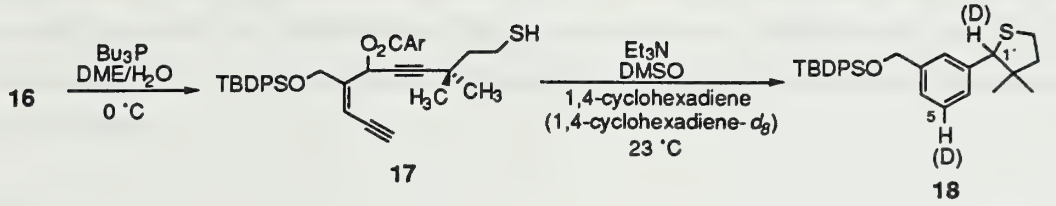


EDAC = 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride
TBDPS = t-Bu(Ph)$_2$Si, Ar = -Ph(NO$_2$)$_2$

Mechanistic Studies of Non-Macrocyclic Analogs

Each of the previously mentioned analogs produced cycloaromatized products upon treatment with a thiol. Cumulene intermediates were observed in the case of **9** and **13** and an intramolecular hydrogen abstraction was found in studies of **10**. The most promising mechanistic studies, however, were carried out on analog **16**. Treatment of disulfide **16** as in Scheme V led to the production of the stable thiol **17**. Subsequent addition of triethylamine to a deoxygenated solution of **17** in DMSO containing 1,4-cyclohexadiene or 1,4-cyclohexadiene-d_8, gave the cycloaromatized product **18** in 75% yield (45% with the deuterio radical trap). These results, as well as the observation of an allene intermediate by ^{1}H NMR, suggest that the products are produced by a mechanism similar to that given in Scheme 1. Further trapping studies in neat methanol also suggest that analog **14** has the propensity for dichotomous (radical versus polar) reactivity.

Scheme V



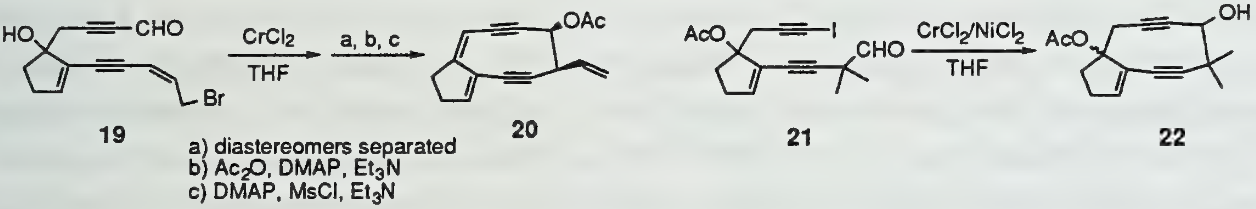
MACROCYCLIC ANALOGS

9-Membered Ring Analogs

As mentioned above, extensive effort has been devoted to the development of synthetic strategies which would allow the facile assembly of the strained cyclononenediyne core of chromophore **1**. As a result, the synthesis of several 9-membered ring analogs have been reported in the literature.

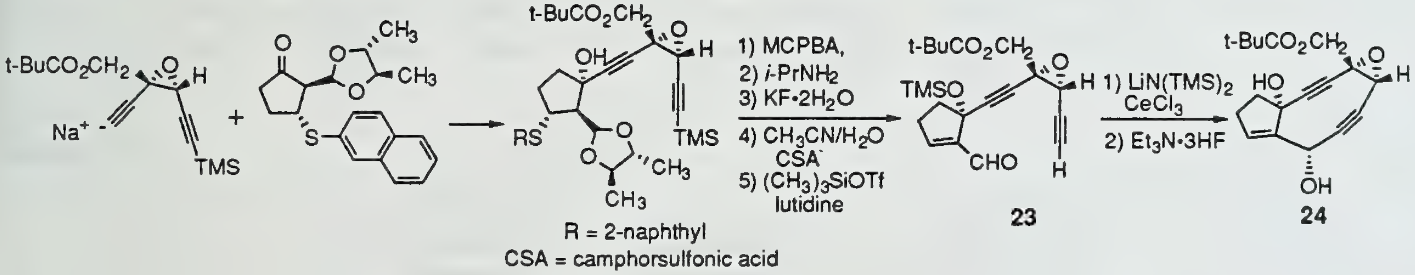
One of the first analogs of this type, **20**, was designed by Wender (Scheme VI).[19] This molecule, as well as Buszek's analog **22** (Scheme VI),[20] underwent ring closure via chromium-mediated cyclizations of **19** and **21**. Although, the precursors to these molecules were synthesized readily, in processes similar to those in Schemes I-III, both reaction schemes unfortunately gave diastereomeric mixtures within the synthesis or as final products.

Scheme VI



However, in 1991, Myers reported the enantioselective synthesis of the epoxy diyne core **24** (Scheme VII). This convergent synthesis, analogous to Scheme IV, incorporated an intramolecular cerium-mediated ring closure of **23**.[21] A diastereomer of **24** was later converted to a functional analog of **1** and was investigated in mechanistic studies.

Scheme VII

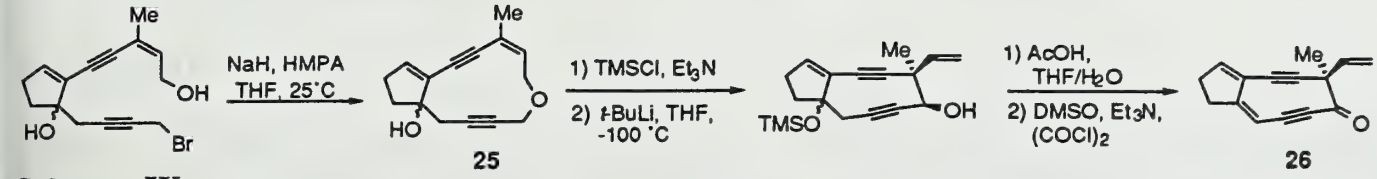


R = 2-naphthyl
CSA = camphorsulfonic acid

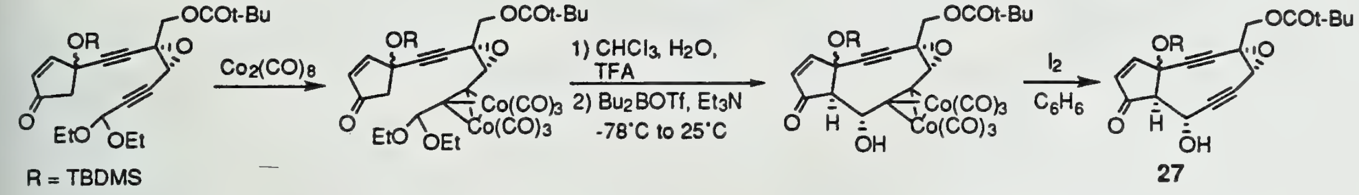
Two other strategies which have proven to be successful in the synthesis of mimics of **1** and other enediyne antibiotic analogs are summarized in Schemes VIII and IX. The racemic synthesis of analog **26**

by Takahashi (Scheme VII),[22] proceeds through a 13-membered ring intermediate **25** that undergoes a [2,3] Wittig rearrangement in the critical step of the reaction scheme. Furthermore, analog **27**, designed by Magnus and coworkers,[23] utilizes an aldol cyclization of a cobalt protected intermediate to construct the strained ring of the mimic.

Scheme VIII



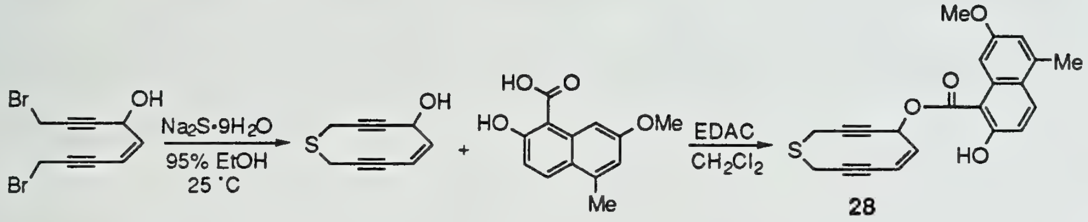
Scheme IX



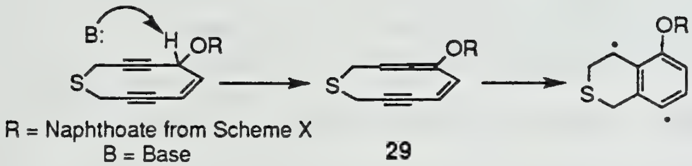
Design and DNA Cleaving Properties of 10-Membered Ring Analogs

The lability of the aforementioned 9-membered ring analogs has prevented their evaluation as potential DNA cleaving agents. However, the synthesis and *in vitro* DNA cleavage properties of 10-membered ring analogs have been reported. One example is Toshima's analog **28** (Scheme X).[24] Interestingly, the naphthoate moiety of **1**, a DNA intercalator,[25] is incorporated into the molecule in the last step of the synthesis. This analog, as well as similar analogs incorporating other intercalating groups, was shown to cleave covalently closed supercoiled ΦX174 DNA to the open circular form at pH > 7. Although the DNA cleavage mechanism is not completely understood, earlier work by Toshima suggests that the mechanism could possibly proceed through the enyne cumulene **29** (Scheme XI) via a Myers-type cyclization similar to that given in Scheme 1.[26]

Scheme X

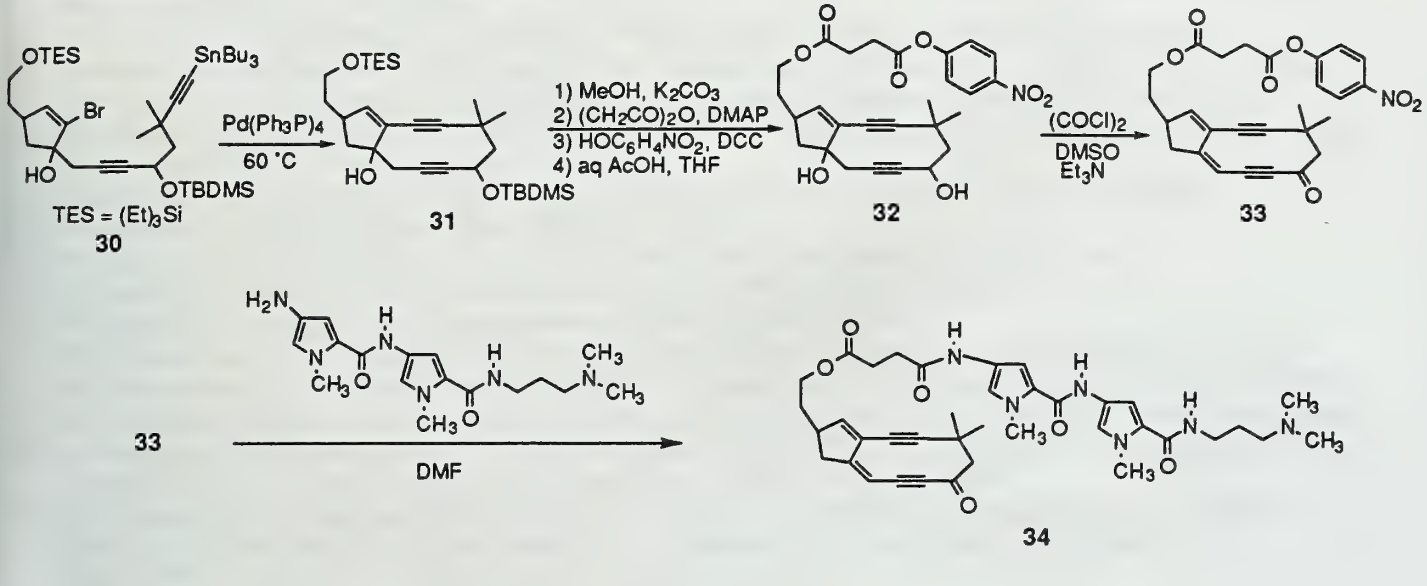


Scheme XI



Similarly, Hirama has described the preparation of several 10-membered ring analogs including **34** which incorporates a netropsin-type DNA minor groove binder (Scheme XII).[27,28] Synthetically, macrocyclic ring closure was realized through an intramolecular Stille coupling of intermediate **30**. The resultant species, **31**, was then converted to the functional analog **34** in several synthetic steps. Furthermore, the racemic analog **34** was shown to cleave DNA more effectively than previous analogs which lack DNA binding moieties.

Scheme XII



CONCLUSIONS

The synthesis and properties of compounds related to the neocarzinostatin chromophore, **1**, have been discussed in this abstract. The synthesis of stable macrocyclic analogs, particularly the 9-membered ring mimics, has proven to be rather difficult due to the highly strained nature of the core. However, macrocyclic, as well as "strain-released" acyclic analogs, have been synthesized and shown to be useful as both mechanistic probes and DNA cleaving agents.

The potential for molecules such as those reported herein, to act *in vivo* depends, as in the case of **1** in the absence of its apoprotein, upon their stability under physiological conditions. Further research in this area should offer insights into the mechanism of action of **1** and will hopefully lead to biologically useful non-natural antitumor agents.

REFERENCES

1) (a) Nicolaou, K. C.; Dai, W. M. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1387-1530. (b) Nicolaou, K. C.; Dai, W. M.; Tsay, S. C.; Estevez, V. A.; Wrasidlo, W. *Science* **1992**, *256*, 1172-1178. (c) Nicolaou, K. C.; Smith, A. L. Yue, E. W. *Proc. Natl. Acad. Sci, U.S.A.* **1993**, *90*, 5881-5888. (d) Maier, M. E. *Synlett* **1995**, *1*, 13-26.
2) Zeng, Z. *Organic Chemistry Seminar Abstracts, University of Illinois* **1988-1989**, *2*, 162-169. (and references therein)
3) (a) Ishida, N.; Miyazaki, K.; Kumagai, K.; Rikimaru, M. *J. Antibiot.* **1965**, *18*, 68-76.

4) holoprotein: (a) Napier, M. A.; Holmquist, B.; Strydom, D. J.; Goldberg, I. H. *Biochem. Biophys. Res. Commun.* **1979**, *89*, 635-642. (b) Koide, Y.; Ishii, F.; Hasuda, K.; Koyama, Y.; Edo, K.; Katamine, S.; Kitame, F.; Ishida, N. *J. Antibiot.* **1980**, *33*, 342-346. apoprotein: (a) Meienhofer, J.; Maeda, H.; Glaser, C. B.; Czombos, J.; Kuromizu, K. *Science* **1972**, *178*, 875-876. (b) Samy, T. S. A.; Hahm, K.; Modest, E. J.; Lampman, G. W.; Keutmann, H. T.; Umezawa, H.; Herlihy, W. C.; Gibson, B. W.; Carr, S. A.; Biemann, K. *J. Biol. Chem.* **1983**, *258*, 183-191.

5) (a) Edo, K.; Mizugaki, M.; Koide, Y.; Seto, H.; Furihata, K.; Otake, N.; Ishida, N. *Tetrahedron Lett.* **1985**, *26*, 331-334. (b) Edo, K.; Ariyama, Y.; Saito, K.; Mizugaki, M. *J. Antibiot.* **1986**, *39*, 1615-1619. (c) Myers, A. G.; Proteau, P. J.; Handel, T. M. *J. Am. Chem. Soc.* **1988**, *110*, 7212-7214.

6) Kim, K.; Kwon, B.; Myers, A. G.; Rees, D. C. *Science*, **1993**, *262*, 1042-1046.

7) (a) Goldberg, I. H. *Acct. Chem. Res.* **1991**, *24*, 191-198. (b) Murphy, J. A.; Griffiths, J. *Nat. Prod. Rep.* **1993**, *10*, 551-564.

8) Charnas, R. L; Goldberg, I. H. *Biochem. Biophys. Res. Commun.* **1984**, *122*, 642-648.

9) Hensens, O. D.; Dewey, R. S.; Liesch, J. M.; Napier, M. A.; Reamer, R. A.; Smith, J. L.; Albers-Schönberg, G.; Goldberg, I. H. *Biochem. Biophys. Res. Commun.* **1983**, *113*, 538-547.

10) (a) Myers, A. G. *Tetrahedron Lett.* **1987**, *28*, 4493-4496. (b) Myers, A. G.; Proteau, P. J. *J. Am. Chem. Soc.* **1989**, *111*, 1146-1147.

11) Myers, A. G.; Harrington, P. M.; Kwon, B. *J. Am. Chem. Soc.* **1992**, *114*, 1086-1087.

12) Bergman, R. G. *Acct. Chem. Res.* **1973**, *6*, 25-31.

13) Myers, A. G.; Cohen, S. B.; Kwon, B. *J. Am. Chem. Soc.* **1994**, *116*, 1670-1682.

14) Fujiwara, K; Sakai, H; Hirama, M. *J. Org. Chem.* **1991**, *56*, 1688-1689.

15) Wender, P. A.; Tebbe, M. J. *Tetrahedron* **1994**, *50*, 1419-1434.

16) (a) Scheuplein, S. W.; Harms, K.; Brückner, R.; Suffert, J. *Chem. Ber.* **1992**, *125*, 271-278. (b) Scheuplein, S. W.; Machinek, R.; Suffert, J.; Brückner, R. *Tetrahedron Lett.* **1993**, 6549-6552.

17) (a) Nakatani, K.; Arai, K.; Terashima, S.*Tetrahedron* **1993**, *49*, 1901-1912. (and references therein)

18) (a) Myers, A. G.; Dragovich, P. S. *J. Am. Chem. Soc.* **1989**, *111*, 9130-9132. (b) Myers, A. G.; Dragovich, P. S.; Kuo, E. Y. *J. Am. Chem. Soc.* **1992** *114*, 9369-9386.

19) (a) Wender, P. A.; Harmata, M.; Jeffrey, D.; Mukai, C.; Suffert J. *Tetrahedron Lett.* **1988**, *29*, 909-912. (b) Wender, P. A.; McKinney, J. A.; Mukai, C. *J. Am Chem. Soc.* **1990**, *112*, 5369-5370.

20) Buszek, K. R.; Jeong, Y. *Synth. Commun.* **1994**, *24*, 2461-2472.

21) (a) Myers, A. G.; Alauddin, M. M.; Fuhry, M. A. M. *Tetrahedron Lett.* **1989**, *30*, 6997-7000. (b) Myers, A. G.; Harrington, P. M.; Kuo, E. Y. *J. Am. Chem. Soc.* **1991**, *113*, 694-695.

22) (a) Doi, T.; Takahashi, T. *J. Org. Chem.* **1991**, *56*, 3465-3467. (b) Takahashi, T.; Tanaka, H; Hirai, Y.; Doi, T.; Yamada, H.; Shiraki, T.; Sugiura, Y. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1657-1659.

23) (a) Magnus, P.; Pitterna, T. *J. Chem. Soc., Chem. Commun.* **1991**, *7*, 541-543. (b) Magnus, P.; Davies, M. *J. Chem. Soc., Chem. Commun.* **1991**, *21*, 1522-1524. (c) Magnus, P. *Tetrahedron* **1994**, *50*, 1397-1418.

24) (a) Nicolaou, K. C.; Skokotas, G.; Maligres, P.; Zuccarello, G.; Schweiger, E. J.; Toshima, K. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 1272-1279. (b) Toshima, K.; Ohta, K.; Ohashi, A.; Ohtsuka, A.; Nakata, M.; Tatsuta, K. *J. Chem. Soc., Chem. Commun.* **1992**, *18*, 1306-1308. (c) Toshima, K.; Ohta, K.; Nakamura, T.; Nakata, M.; Matsumura, S. *J. Chem. Soc., Chem. Commun.* **1993**, *19*, 1525-1527.

25) Povirk, L. F.; Dattagupta, N.; Warf, B. C.; Goldberg, I. H. *Biochemistry*, **1981**, *20, 4007-4014.*

26) (a) Toshima, K.; Ohta, K.; Ohtake, T.; Tatsuta, K. *Tetrahedron Lett.* **1991**, *32*, 391-394. (b) Toshima, K.; Ohta, K.; Ohtsuka, A.; Matsumura, S.; Nakata, M. *J. Chem. Soc., Chem. Commun.* **1993**, *18*, 1406-1407. (c) Toshima, K.; Ohta, K.; Kano, T.; Nakamura, T.; Nakata, M.; Matsumura, S. *J. Chem. Soc., Chem. Commun.* **1994**, *19*, 2295-2296. (d) Toshima, K.; Koji, Y.; Ohta, K.; Kano, T.; Nakata, M. *Tetrahedron Lett.* **1994**, *35*, 1573-1576.

27) (a) Hirama, M.; Fujiwara, K.; Shigematsu, K.; Fukazawa, Y. *J. Am. Chem. Soc.* **1989**, *111*, 4120-4122. (b) Fujiwara, K.; Kurisaki, A.; Hirama, M. *Tetrahedron Lett.* **1990**, *31*, 4329-4332. (c) Hirama, M.; Tokuda, M.; Fujiwara, K. *Synlett* **1991**, 651-653. (d) Hirama, M.; Gomibuchi, T.; Fujiwara, K. *J. Am. Chem. Soc.* **1991**, *113*, 9851-9853. (e) Kawata, S.; Oishi, T.; Hirama, M. *Tetrahedron Lett.* **1994**, *35*, 4595-4598.

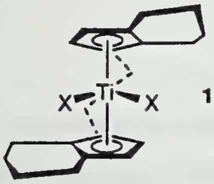
28) Masahiko, T.; Fujiwara, K.; Gomicuchi, T.; Hirama, M.; Uesugi, M.; Sugiura, Y. *Tetrahedron Lett.* **1993**, *34*, 669-672.

SYNTHESIS AND ASYMMETRIC REACTIVITY OF BRIDGED *ANSA*-BIS(INDENYL)-BASED CHIRAL TITANOCENES

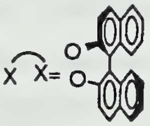
Reported by Haibo Jiang   March 6, 1995

INTRODUCTION

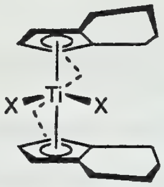
Group IV *ansa*-bis(indenyl) ligand-based chiral metallocenes have recently been regarded with intense interest as potential mediators of enantioselective transformations.[1] The application of an alkyl- or ring-bridged bis(indenyl) ligand as a particularly stereorigid support[2] for introducing chirality is uniquely attractive due to the superior tenacity with which it attaches itself to transition metals, involving bond strengths as high as 118 kcal/mol.[3] This abstract will focus on bridged *ansa*-bis(indenyl)-based chiral titanocenes. The most effective of the enantioselective reagents are those that employ the ethylene bis(tetrahydroindenyl) (EBTHI) in the chiral complexes of type **1**.[4]



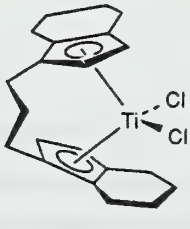
1a: X=Cl, racemic



1b; X X =



1c: X=Cl, meso



2

Historically, the initial interest in bridged *ansa*-bis(indenyl)-based chiral titanocenes was mainly focused on their synthesis, resolution, and physical properties. Recently, many asymmetric reactions in organic syntheses have been reported. Reactions mediated by these titanocenes can be divided into three main areas: (1) catalytic asymmetric reactions including hydrogenation of C=C and C=N bonds, hydrosilylation of ketones, epoxidations[5] and olefin isomerizations;[6] (2) stoichiometric diastereoselective reactions such as enantioselective allylation of aldehydes; (3) catalytic polymerization of alkenes into stereoregular polymers.[7] The main focus of this abstract will be the synthesis of these chiral titanocenes, and the catalytic hydrogenation of C=N bonds, ketone hydrosilylation and enantioselective allylation of aldehydes as well as their mechanisms.

SYNTHETIC ASPECTS

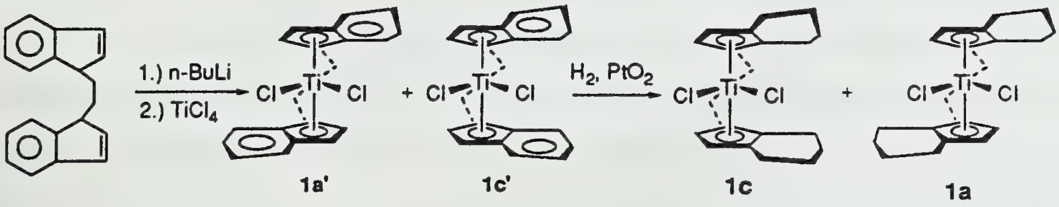
Chiral titanocenes can be obtained in two general ways, metalation of chiral or prochiral cyclopentadienyl-based ligands or convertion of achiral cyclopentadienyl-based titanocenes via appropriate functionalization into chiral derivatives. Since Brintzinger's[4a] first report in 1982 of the metalation of ethylene-bridged *ansa*-bis(indenyl) with $TiCl_4$ to form *ansa*-titanocene **1**, the preparation of this type of bridged *ansa*-bis(indenyl)-based chiral titanocene and its derivatives by metalation has dominated the field of chiral titanocenes.

Copyright © 1995 By (Haibo Jiang)

Ethylene-Bridged Bis(tetrahydroindenyl) Titanocene [(EBTHI)$TiCl_2$]

Brintzinger[4a] reported the synthesis of the first bridged bis(indenyl) chiral titanocene (Scheme I). The metalation of 1,2-bis(indenyl)ethane with $TiCl_4$ followed by reduction results in a mixture of **1a**(*racemic*) and **1c**(*meso*). The ratio of **1a** to **1c** is greatly improved by converting **1c** into **1a** by exposure to sunlight or irradiation with a mercury lamp. The *racemic* complex could then be derivatized into a single enantiomeric alkoxide (*S*,*S*)-1:(*S*)-1'-**1b** upon treatment with (*S*)-1,1'-bi-2-naphthol by kinetic resolution and the alkoxide could be separated by chromatograpgy. Treatment of **1b** with HCl regenerated enantiomerically pure C_2-symmetrical titanocene (*S*,*S*)-**1a**.

Scheme I



$$\mathbf{1c} \xrightarrow{hv} \mathbf{1a} \xrightarrow{(S)\text{-}1,1'\text{-bi-2-naphthol, Na}} \mathbf{1b} \xrightarrow{HCl} (S,S)\text{-}\mathbf{1a}$$

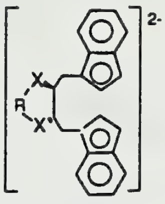
In order to simplify the resolution of *racemic* **1a**, Brintzinger[4b] reported that *O*-acetyl-*R*-mandelic acid gave a better resolution than binaphthol. Collins[4c] also improved the synthetic yield of **1a'**and **1c'** from 22% to 50% by slow addition of dilithio-1,2-bis(indenyl)ethane and $TiCl_4$ to THF simultaneously with vigorous stirring.

To this point, the synthetic strategies involved the initial formation of an isomeric mixture of *racemic* and *meso* bis(indenyl) titanocene, which first must be separated, followed by optical resolution of the enantiomers as diastereomeric derivatives, and finally conversion of these derivatives back into the enantiomerically pure complexes. Because resolution is unpredictable and time consuming, and requires large quantities of materials, attempts were made to circumvent these problems.

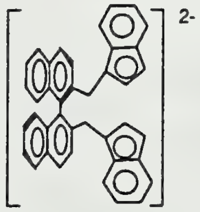
One approach employed by Brintzinger[8] has been to increase the bridge strap to three carbon atoms. A synthetic route similar to that to **1** was used and titanocene **2** was obtained solely as the racemate. Although this solved the separation of *racemic* and *meso* isomers, the complex was no longer C_2-symmetric because the inherent tilt angle of the Cp rings accomodate the larger length of the strap by rotating the Cp rings.[9] The C_1 structure makes the two coordination positions bearing the X groups sterically nonequivalent so that stereoselective reactions involving these sites will be controlled by different steric environments, leading to ambiguity in defining the origins of the stereoselection.

Many other attempts were made to avoid resolution by introducing chiral centers into the strap. The idea behind this design is the expectation that the chirality of the strap would induce a

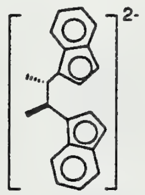
single enantiomeric titanocene and thus avoid the resolution step. Six examples of such ligands are shown: **3a**,[10] **3b**,[11] **4**,[12] **5**,[13] **6**,[6] **7**.[5] Each of these ligands proved to have its own difficulties.



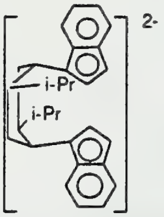
3a: X=O, R=Me_2C
3b: X=CH_2, R=CH_2



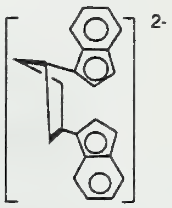
4



5



6

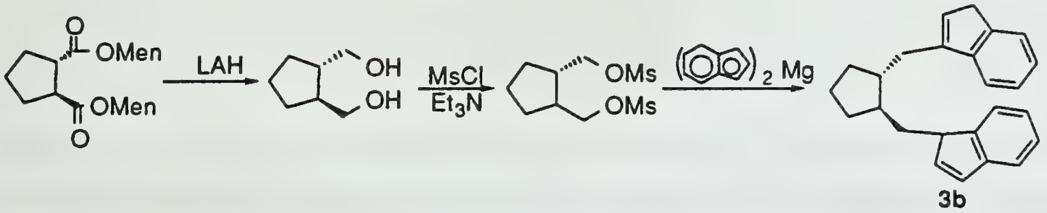


7

(*S*,*S*)-[Ti((*R*,*R*)-Cyclacene)Cl_2]

Ligand **3b** was obtained in 40% overall yield from (-)-dimenthyl succinate and afforded (*S*,*S*)-[Ti((*R*,*R*)-cyclacene)Cl_2] upon complexation and reduction (Scheme II).[11] No other monomeric isomers of the bis(indenyl) or the cyclacene complex were detected by ^{1}H NMR spectroscopy. However, it was not a C_2-symmetric complex.

Scheme II



Ligand **3a** possesses an acid-sensitive acetonide group, and only the *meso* isomer was isolated. Whereas ligand **4** suppresses the formation of an undesired *meso* titanocene and favors the formation of the single *racemic* complex, the complex is not C_2-symmetric. Ligand **5** does give a C_2-symmetric complex, but its Ti(IV) dichloride adduct consists of two diastereomers of the *racemic* form and substantial amounts of the "*meso*" isomer. Halterman also reported that ligands **6** or **7** could be produced as a single *racemic* diastereomer but lack C_2-symmetry.

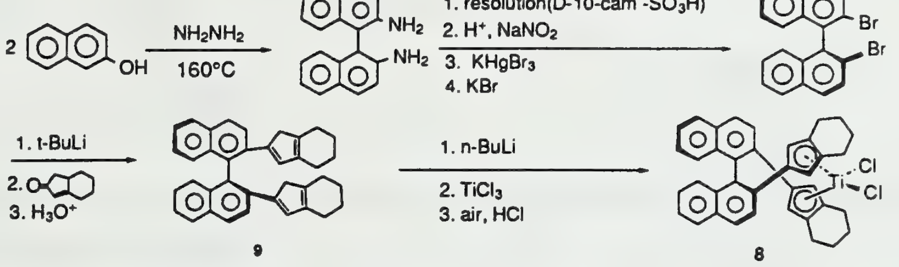
[Ti(*R*,*S*)-Binapacene)Cl_2]

Very recently, groups led by Halterman[5b] and Bosnich[14] independently synthesized the enantiopure C_2-symmetric complex [Ti((*R*,*S*)-binapacene)Cl_2], **8**. It is the first reported enantiomerically enriched C_2-symmetrical bridged titanocene which contains a stereogenic bridge linking the two indenyl groups.

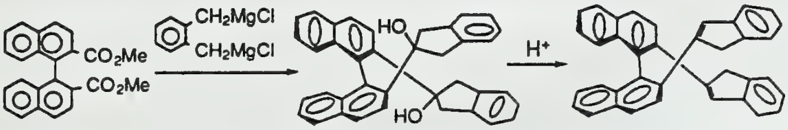
In Halterman's report, the synthesis of the enantiomerically enriched binaphthalene derived bis(indene) ligand **9** for the preparation of chiral metallocene **8** is straightforward and proceeds through 1,1'-binaphthalene-2,2'-diamine[15] (Scheme III). Since the two faces of the cyclopentadienyl ligands are homotopic, metalation of **9** yields only a single enantiopure C_2-symmetrical complex **8**. Bosnich[14] used a similar procedure to prepare the binapacene complex **8** from the racemic diester (Scheme IV). Compared with Halterman's route, Bosnich's

approach proceeds through a diester, whose preparation is more complicated, the yield of complexation was also much lower.[16]

Scheme III



Scheme IV



Bosnich[14] also prepared two analogue of the desired complex **8**. Both systems gave only a single isomer of each complex, and the tetrahydroindenyl ligands project their C_2 chirality directly into the site of reaction.

Although more and more interest has been focused on applications using bridged bis(indenyl) chiral titanocenes and on zirconocene chemistry, novel bridged C_2-symmetrical titanocenes are still under exploration. For example, Buchwald[17] reported the synthesis of a chiral, doubly bridged *ansa*-titanocene complex. The more sterically rigid double bridge precludes the formation of *meso* or C_1 diastereomers or conformers.

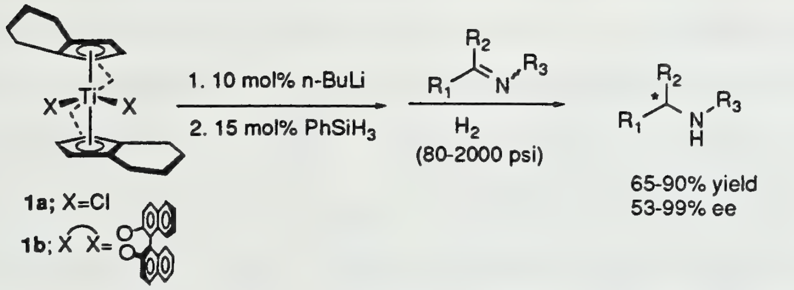
ASYMMETRIC REACTIVITIES

Catalytic Asymmetric Hydrogenation of Imines and Enamines

Buchwald used (EBTHI)TiX_2, **1**, the most widely and successfully used chiral metallocene, as the precatalyst in the catalytic asymmetric hydrogenations of imines[18] and enamines.[19] On reaction of **1a** or **1b** with 2 equiv of n-BuLi in THF, followed by 2.5-3 equiv of phenylsilane, an active hydrogenation catalyst was formed. This active species reacted with the imine to afford the amine in good to excellent ee (Scheme V).[18]

The reaction is believed to proceed through a titanium(III) hydride species,[20] though its exact nature remains unclear, partly because solutions of this species are extremely air sensitive and exhibit no resonances in their ^1H NMR spectra. Brintzinger[21] has provided EPR evidence that reaction of titanocene dichloride with alkyl Grignard or alkyllithium reagents forms a titanium(III) hydride, although at temperatures above -20°C this complex was unstable. Harrod and Samuel[22] also reported that EPR spectra showed the existence of a mixture of paramagnetic Ti(III) after reaction of titanocene **1** with methyl Grignard reagent followed by addition of phenylsilane. The

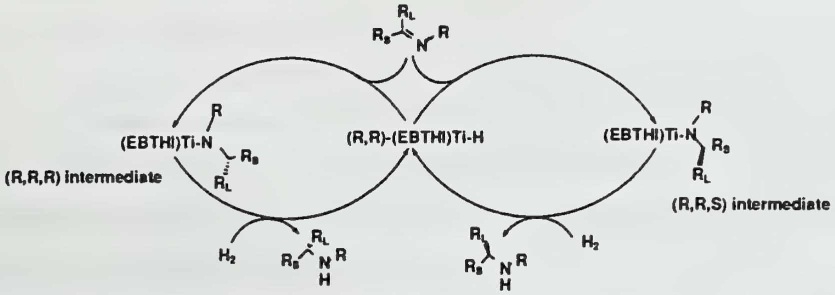
Scheme V



predominant signal was characteristic of a titanium(III) silyl complex in which hydride functions as a bridge linking two Ti(III) atoms.

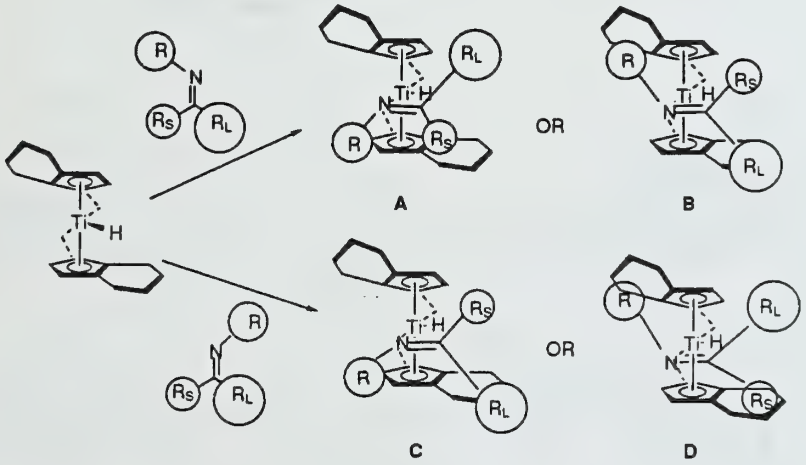
Buchwald[18a] proposed the catalytic cycles shown in Scheme VI. The first step involves 1,2-insertion of the imine into the Ti(III) hydride to form two diastereomeric titanium amide complexes. The second step is hydrogenolysis of these amides, via a sigma bond metathesis process, to form enantiomeric amines and regenerate the Ti(III) hydride.

Scheme VI



In order to account for the enantioselectivity, Buchwald proposed a stereochemical model (Scheme VII). First, although initial coordination of the imine likely occurs via the lone pair of electrons on nitrogen, the geometries shown are required in order to allow proper overlap of the imine π* orbital with the Ti-H sigma orbital.[23] The alkyl substituent on nitrogen plays a dominant

Scheme VII



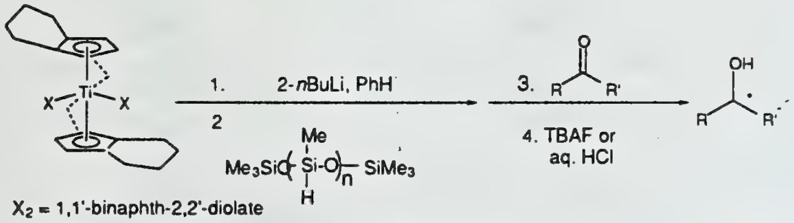
role in controlling the stereochemical outcome of the reaction. In transition state **B**, steric interactions between R_L and the tetrahydroindenyl ligand(THI), as well as between R and THI, are present. In A, only the interaction between R_S and THI is present, making A more favorable than B. Thus, with an *anti* imine, the (*R,R*) catalyst **1** would be expected to afford the (*R*) enantiomer of the product. The same should hold for a *syn* imine: because of the dominant nitrogen substituent, **C** is expected to be more favorable than is **D**. Therefore, the (*S*) enantiomer is expected for *syn* imine with catalyst (*R,R*) **1**. The experimental results match the prediction very well.[18a,b]

The other experimental result, that a cyclic imine gives a higher enantioselectivity and higher rate than an acyclic imine, can also be explained by the above model. In the titanium amide intermediate formed from the reaction of **1** with a cyclic imine, both the nitrogen and the substituents are "tied back" and therefore react more rapidly with hydrogen. The higher ee is probably due to the fact that cyclic imines can only exist as a single isomer, while acyclic imines are usually mixtures of *E* and *Z* isomers.[24]

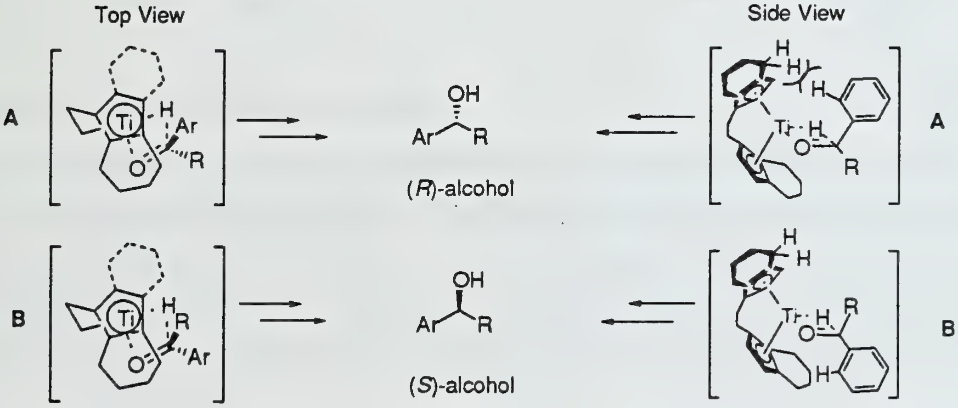
Enantioselective Hydrosilylation of Ketones

The type **1** titanocene complex was also employed by Buchwald[25] to reduce ketones through an asymmetric hydrosilylation pathway (Scheme VIII). The system can reduce aromatic or conjugated ketones with excellent ee, but with saturated ketones, ee's are only moderate. It is proposed by Buchwald and Carter[25] that a similar stereochemical model for hydrogenation of imines is still applicable to hydrosilylation (Scheme IX).

Scheme VIII



Sheme IX

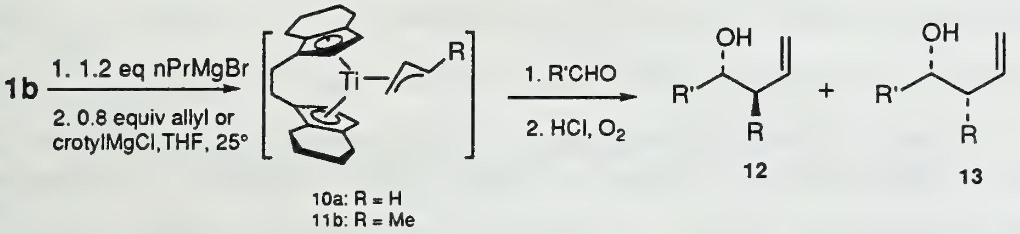


In Scheme IX, the ketone approaches the titanium complex from the side. If one assumes that the aromatic (or olefin) group and the carbonyl group remain in conjugation throughout the reaction[26], the aromatic group should not rotate to minimize interactions with the catalyst. The ketone thus prefers to approach the complex in transition state **B** to minimize steric interaction between the cyclohexyl portion of the tetrahydroindenyl ligand and the aromatic ring of the ketone. Reaction through transition state **B** results in the (*S*)-alcohol with high ee as observed. For unconjugated ketones, rotation of the side chain can occur upon approach to the catalyst to minimize steric interaction, and thus the ee of the reaction is low.

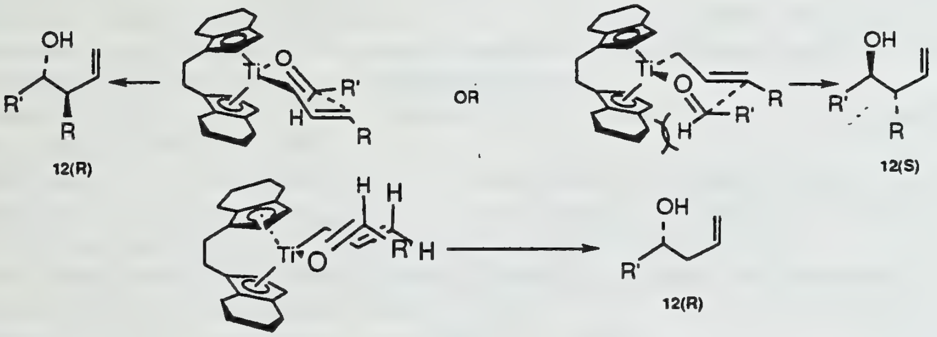
Diastereomeric and Enantioselctive Allylation of Aldehydes

Homochiral allyl- and crotyltitanocene **10a** (R=H) and **10b** (R=Me) prepared from *ansa*-titanocene **1** was used by Collins[27] to react with aldehydes, to provide homoallylic alcohols. In the case of **10b,** anti diastereoselectivity was moderate to excellent as shown in Scheme X. The enantioselectivity of this process is modest and appears to arise from product formation via competing formation of chair- and boat-like transition states (Scheme XI).

Scheme X



Scheme X



CONCLUSION AND FUTURE WORK

With the increasing interest in searching for good enantioselective catalysts, a growing number of novel bridged *ansa*-bis(indenyl) chiral titanocenes have been synthesized, and their applications in asymmetric reactions have been investigated. With the success of zirconium analogue in catalyzing asymmetric syntheses such as Diels-Alder and aldol reactions, the chiral titanocenes are expected to perform in such transformation as well. In addition, the antitumor

properties of biscyclopentadienyl based titanocenes is a good sign for future development of novel titanocenes in drug design.[28]

REFERENCES

1. (a) Halterman, R. L. *Chem. Rev.* **1992**, 92, 965. (b) Wagener, K. B. *Science* **1995**, *267*, 191. (c) Coates, G. W.; Waymouth, R. M. *Science* **1995**, *267*, 217. (d) Doman, T. N.; Hollis, T. K.; Bosnich, B. *J. Am. Chem. Soc.* **1995**, *117*, 1352. (e) Gupta, V. K.; Stash, S.; Bhardwaj, I. S. *J. M. S. Rev. Macromol. Chem. Phys.* **1994**, C34(3), 439.
2. Schnutenhaus, H.; Brintzinger, H. H. *Angew. Chem.* **1979**, *91*, 837.
3. Jacobson, D. B.; Frieser, B. S. *J. Am. Chem. Soc.* **1985**, *107*, 7399.
4. (a) Wild, F.R.W.P.; Zsolnai, L.; Huttner, G.; Brintzinger, H. H. *J. Organomet. Chem.* **1982**, *232*, 233. (b) Schäfer, A.; Karl, E.; Zsolnai, L.; Huttner, G.; Brintzinger, H. H. *J. Organomet. Chem.* **1987**, *328*, 87. (c) Collins, S.; Kuntz, B. A.; Taylor, N. J.; Ward, D. G. *J. Organomet. Chem.* **1988**, *342*, 21.
5. (a) Halterman, R.L.; Ramsey, T.M. *Organometallics* **1993**, *12*, 2879. (b) Colletti, S. L.; Halterman, R. L. *Tetrahedron Lett.* **1992**, *33*, 1005.
6. Chen, Z.; Halterman, R. L. *J. Am. Chem. Soc.* **1992**, *114*, 2276.
7. For reviews see: Gupta, V. K.; Satish, S.; Bharwwaj, I. S. *J. M. S.-Rev. Macromol. Chem. Phys.* **1994**, C34(3), 439.
8. RÖll, W.; Zsolnai, L.; Huttner, G.; Brintzinger, H. H. *J. Organomet. Chem.* **1987**, *322*, 65.
9. Smith, J. A.; Seyerl, J. V.; Huttner, G.; Brintzinger, H. H. *J. Organomet. Chem.* **1979**, *173*, 175.
10. Bandy, J. A.; Green, M. L. H.; Gardiner, I. M.; Prout, *K. J. Chem. Soc., Dalton Trans.* **1991**, 2207.
11. Hollis, T. K.; Rheingold, A. L.; Robinson, N. P.; Whelan, J.; Bosnich, B. *Organometallics* **1992**, *11*, 2812.
12. Burk, M.; Colletti, S. L.; Halterman, R. L. *Organometallics* **1991**, *10,* 2998.
13. Rheingold, A. L.; Robinson, N.P; Whelan, J.; Bosnich, B. *Organometallics* **1992**, *11*, 1869.
14. Ellis, W. W.; Hollis, T. K.; Odenkirk, W.; Whelan, J.; Ostrander, R.; Rheingold, A. L.; Bosnich, B. *Organometallics* **1993**, *12*, 4391.
15. Brown, K. J.; Berry, M. S.; Murdoch, J. R. *J. Org. Chem.* **1985**, 50, 4345.
16. Conventional complexation of **8'** gave a number of unexpected results, e.g. formation of a cyclic compound between two cyclopentadienyl ring.
17. Grossman, R. B.; Tsai, J.-C.; Davis, W. M.; Gutiérrez, A.; Buchwald, S. L. *Organometallics* **1994**, *13*, 3892.
18. a) Willoughby, C. A.; Bulchwald, S. L. *J. Am. Chem. Soc.* **1994**, *116*, 11703. (b) Willoughby, C. A.; Bulchwald, S. L. *J. Am. Chem. Soc.* **1992**, *114*, 7562.
19. Lee, N. E.; Buchwald, S. L. *J. Am. Chem. Soc.* **1994**, *116*, 5895.
20. For other examples of Ti(III) hydride see following reference and thereafter: Martin, H. A.; Jellinek, F. *J. Organomet. Chem.* **1968**, *12*, 149.
21. Brintzinger, H. H. *J. Am. Chem. Soc.* **1968**, *89*, 6871.
22. (a) Aitken, C. T.; Harrod, J. F.; Samuel, E. *J. Am. Chem. Soc.* **1986**, *108,* 4059. (b) Samuel, E.; Mu, Y.; Harrod, J. F.; Dromzee, Y.; Jeannin, Y. *J. Am. Chem. Soc.* **1990**, *112*, 3435.
23. Laugher, J. W.; Hoffman, R. *J. Am. Chem. Soc.* **1976**, *98* , 1729.
24. Willoughby, C.A.; Buchwald, S. L. *J. Org. Chem.* **1993**, *58*, 7627.
25. Carter, M. B.; Schiøh, B.; Gitierrez, A.; Bulchwald, S. L. *J. Am. Chem. Soc.* **1994**, *116*, 11667-11670.
26. The RHF/6-31G* barrier for rotation of the carbonyl group of acetophenone was calculated to be 6.5 kcal/mol (Petillo, P. A. Unpublished results).
27. Collins, S.; Kuntz, B. A.; Hong, Y. *J. Org. Chem.* **1989**, *54*, 4154.
28. KÖpf-Maier, P.; KÖpf, H. In *Structure and Bonding*; Springer: Berlin, New York, 1988; pp 103-185, and references therein.

UNIVERSITY OF ILLINOIS-URBANA

3 0112 027829461

Q.547
I ℓ 6s
1995-96:1

ORGANIC SEMINAR ABSTRACTS
1995-96, SEMESTER I

University of Illinois

Department of Chemistry
Box 68 Roger Adams Laboratory
600 South Matthews Avenue
Urbana, Illinois 61801

January, 1996

Copyright © by the Board of Trustees of the University of Illinois

NOTICE: **Return or renew all Library Materials! The *Minimum* Fee for each Lost Book is $50.00.**

The person charging this material is responsible for its return to the library from which it was withdrawn on or before the **Latest Date** stamped below.

Theft, mutilation, and underlining of books are reasons for disciplinary action and may result in dismissal from the University.
To renew call Telephone Center, 333-8400

UNIVERSITY OF ILLINOIS LIBRARY AT URBANA-CHAMPAIGN

CHEMISTRY LIBRARY

JUN 18 1996

L161—O-1096

SEMINAR TOPICS

Semester I, 1995-96

Page

The Baylis-Hillman Reaction.....1
Alexander Hurd

The Asymmetric Heck Reaction.....9
Zhicai Wu

Mechanism of the Enantioselective Epoxidation of Unfunctionalized Olefins Catalyzed by (Salen)Manganese(III) Complexes.....17
Gerald Weisenburger

Asymmetic Enzymatic Catalysis With Cross-Linked Enzyme Crystals (CLECs).....25
Bryan Yeung

Chromium(0) Mediated Higher-Order Cycloadditions.....33
Mark Seierstad

Comparison of Methodologies Leading to 1-Arylnaphthalene Lignan Lactones.....41
Michael E. Koscho

Acatalytic Asymmetric Hydrogenations Using NovelC_2-Symmetric Bis(Phospholane) Ligands.....49
Marc Skaddan

Preparation of Functionalized Dialkylzincs and Their Catalytic Asymmetric Additions to Aldehydes.....57
Shaun Stauffer

Living Free Radical Polymerization.....65
Peggy-Jean Prest

Polymer Supported Combinational Synthesis of Small Organic Molecules.....73
Samir D. Serrano

Radical Cation Cycloadditions.....81
Zhengguo Zhu

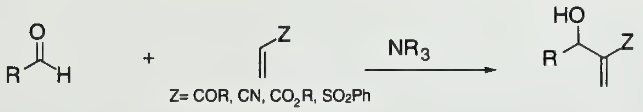
THE BAYLIS-HILLMAN REACTION

Reported by Alexander Hurd September 18, 1995

INTRODUCTION

The Baylis-Hillman reaction is a tertiary amine catalyzed coupling of two reactive species, an aldehyde and a Michael acceptor. This transformation results in a carbon carbon bond forming between the carbonyl of the aldehyde and the α position of the Michael acceptor (Scheme 1).[i,ii] The resulting α-(hydroxyalkyl)acrylates, or Baylis-Hillman adducts, are versatile precursors allowing for the rapid assembly of multifunctional organic compounds under mild conditions. Interestingly, the power and applicability of this reaction has only recently been noticed. Although Baylis and Hillman patented this reaction in 1972,[2] efforts to assess the scope and mechanistic complexity, as well as, to exploit the asymmetric potential of this reaction did not begin for nearly 10 years. Since its rediscovery these adducts or derivatives have found applications as synthons for necic acids,[iii] in the synthesis of α-methylene-γ-butyrolactones,[iv] and in the synthesis of stereodefined trisubstituted olefins.[v] Derivatives of Baylis-Hillman adduct display good to excellent diastereoselectivities in hydrogenations,[vi] epoxidations,[vii] dihydroxylations,[viii] 1,3 dipolar cycloadditions,[ix] and radical addition reactions.[x] The development of an asymmetric Baylis-Hillman reaction using chiral tertiary amines[xi], or chiral acrylates,[11a,xii] has been successful in limited cases. This review will highlight the breadth of this reaction, illustrate the utility of this reaction, and analyze efforts toward an asymmetric variant.

SCHEME I



MECHANISM

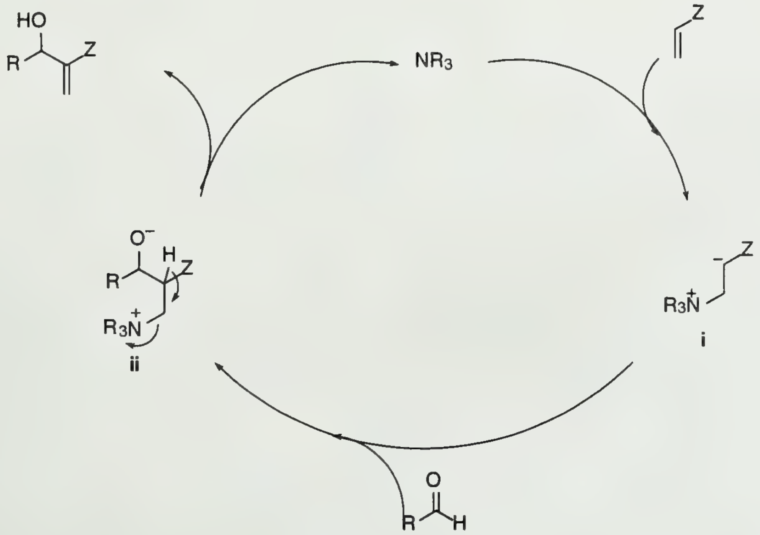
The generally accepted mechanism is viewed as a reversible 1,4 addition of the tertiary amine catalyst to the Michael acceptor, generating a zwitterionic intermediate enolate (**i**) which is attacked by the electrophilic aldehyde. This coupling step has been shown to be the rate determining.[xiii] The zwitterionic coupled intermediate (**ii**) next expels the tertiary amine in either an E2 or E1cB[xiv] mechanism, regenerating the double bond and releasing the tertiary amine to continue the catalytic cycle (Scheme 2). The reversibility of the carbon carbon bond forming step of this reaction is still under debate, however, recent evidence from crossover experiments points to a thermodynamic equilibrium between reacting species and product.[14,xv]

The reaction is slow by most standards, frequently requiring days or occasionally weeks for

Copyright © 1995 by Alexander R. Hurd

completion. Methods for increasing the reactions rate include 1) high pressures,[13bcd] 2) employing 3-hydroxyquinuclidine as the catalyst,[xvi] 3) polar solvents,[xvii] 4) aromatic aldehydes complexed to $Cr(CO)_3$,[xviii] 5) microwave irradiation,[xix] and 6) sonication.[xx] By far high pressure has the greatest influence on the rate of reaction shortening reaction times from weeks to hours and from days to minutes. Pressure dependent kinetic experiments have shown that the Baylis-Hillman reaction has an enormous negative volume of activation, accounting for the high pressure acceleration.[xxi]

SCHEME II



CONSTRUCTION OF THE BAYLIS-HILLMAN ADDDUCT

The Michael Acceptor

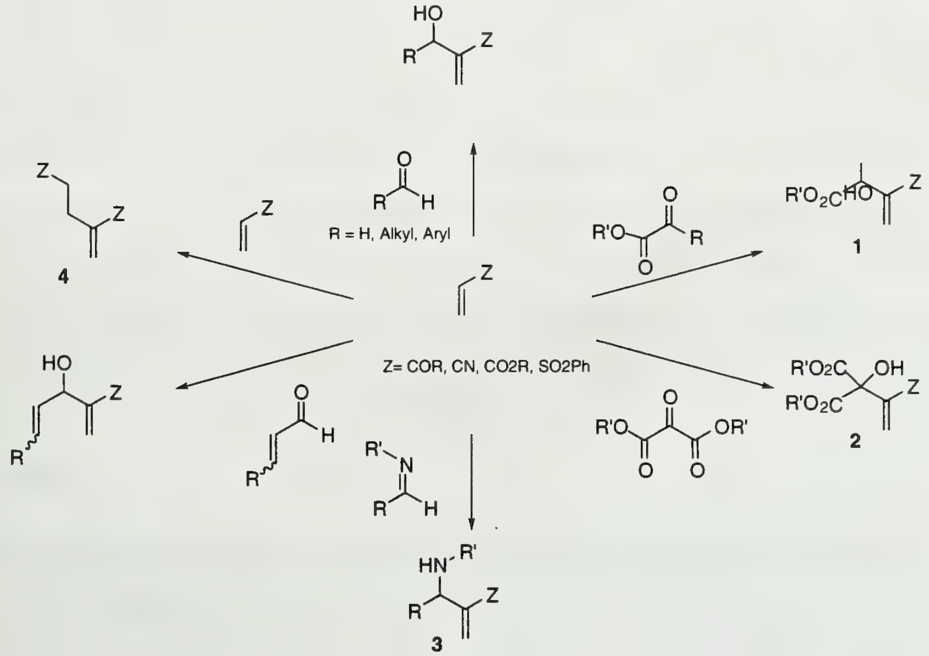
There is a wide range of michael acceptors which have been employed successfully in this reaction. The most common michael acceptors are vinyl ketones, acrylic acid esters, acrylonitrile and vinyl sulfones. However, vinylsulfoxides[6h] and β-allenic esters[xxii] have also been used with some success. Limitations in reactivity do exist. Acrolein is too activated and polymerizes under reaction conditions,[13ad] and unsaturated amides are too deactivated for practical purposes.[13d] The order of reactivity of the michael acceptor is vinyl ketone > acrylonitrile > acrylic ester > vinylsulfone > vinyl sulfoxide.[13d,xxiii] β-Substituted Michael acceptors are generally unreactive, although a few examples with high pressures[13c, 14] or catalysis with DBU[xxiv] exist.

The Electrophilic Component

Aldehydes are the most widely used acceptor moiety. Activated ketones have been reported to react under standard conditions, but the employment of unactivated ketones necessitates high pressure reaction conditions. Scheme III highlights the various electrophiles which have been used in the Baylis-Hillman reaction. Aliphatic, aromatic, and vinyl aldehydes,[1,2] as well as, formaldehyde[4a,13d] react favorably in good to excellent yield. The use of activated ketones opens avenues for further functionalization. For

example, α-keto esters[xxv] and diethylketomalonate.[xxvi] provide the adducts **1** and **2**, respectively. Electrophilic imines allows for rapid construction of an amino analog (**3**).[xxvii] Unsaturated aldehydes will give 1,2 addition products, provided they are β–substituted.[xxviii] In the absence of a reactive electrophile, acrylonitrile and acrylic esters can also function as both the electrophileand the Michael acceptor, adding 1,4 to give α-methylene-1,5-difunctional molecules (**4**) in good yields.[xxix]

SCHEME III



The Catalysts

The function of the catalyst twofold. It must be nucleophilic to function as a michael donor, yet sufficiently nucleofugal for catalysis. The most striking feature of the more effective catalysts is the presence of a highly nucleophilic bridgehead bicyclic nitrogen. However, it is not paramount, since acyclic tertiary amine have also catalyzed this reaction.[13d] DABCO (**5**) is by far the most commonly used catalyst for this transformation. Some tertiary amines which have catalyzed this reaction are depicted in Figure 1.[1,11,24]

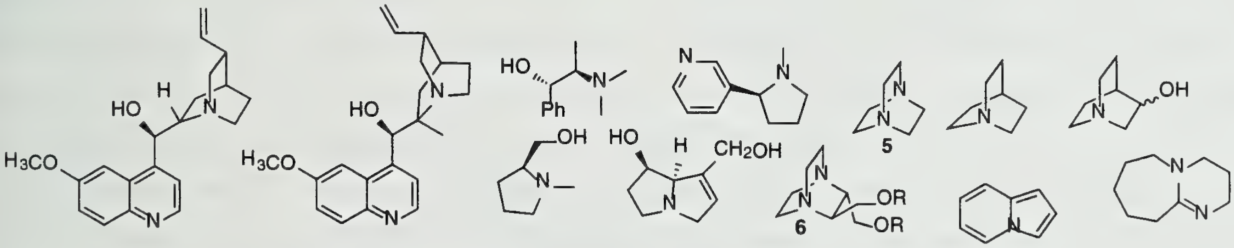


Figure 1. Selected Catalysts in The Baylis-Hillman reaction.

While catalytic loadings (5% -30%) are all that is necessary, in some cases up to stoichiometric amounts often ensure faster reaction. Chiral pool catalysts have also been employed, but the resulting yield

and enantiomer excess are poor.[1,11] Phosphorous catalysts have been used sporadically for reactions with aldehydes and for intramolecular reactions with ketones.[1,15a,27b, xxx]

APPLICATIONS OF THE BAYLIS-HILLMAN ADDUCT

Many strategies for the assemblage of acrylate moieties have been developed to meet the interest in a number of natural products and biologically interesting compounds.[5c] Most, however, require either harsh conditions or multiple functional group manipulations to reveal the acrylate. The Baylis-Hillman reaction, on the other hand, offers a mild, simple, and inexpensive alternative. The dense functionality present in the Baylis-Hillman adduct allows for a variety of controlled manipulations, many of which have recently been reported. The mild conditions of the Baylis-Hillman reaction have allowed the synthesis of otherwise elusive compounds. For example, Hoffman has completed an excellent study of α-methylene-β-keto sulfones to demonstrate the synthesis and the remarkable reactivity of the doubly activated methylene.[23] A discussion of the application of the Baylis-Hillman adducts to multifunctional olefins and examples of diastereoselective manipulations of the adduct will be given.

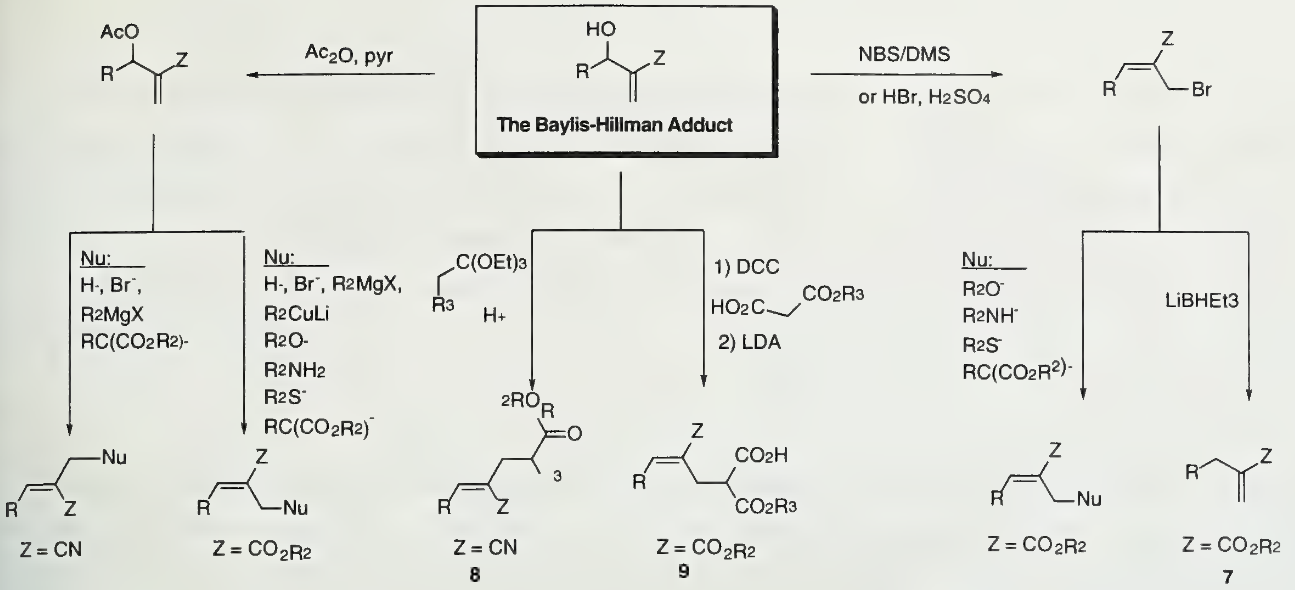
Multifunctional olefins

Scheme IV summarizes the reactivity the Baylis-Hillman adducts with nucleophiles. Conversion to the allyl bromide is facile.[1] Ethyl-α-(bromomethyl)acrylate has been used extensively for the synthesis of α-methylene-γ-butyrolactones via Reformatsky reaction with ketones or aldehydes.[4] Previous reported syntheses are long, tedious, expensive, and/or low yielding.[xxxi] The Baylis-Hillman reaction provides this useful synthon in two high yielding steps. Nucleophilic attack of the allyl bromide usually occurs in an S_N2 manner.[1,5ajk] An exception is the superhydride reduction of acrylic ester bromides which occurs with allylic inversion providing **7**.[5c] Note that this provides the equivalent to the Baylis-Hillman adduct with alkylating agents. Standard Baylis-Hillman conditions prohibit this reaction, as alkylating agents will quaternize the catalytic tertiary amine.

Reaction of allylic acetates with nucleophiles occurs with allylic inversion.[1] Basavaiah and van Heerden independently have demonstrated an interesting dependence of the resulting olefin geometry with judicious choice of the Michael acceptor. Acrylic ester acetates yield trans olefins while acrylonitrile acetates provide the cis isomer preferentially.[5defg] Alternatively, the 2-alkylidene 1,5 difunctional molecules **8** and **9** can also be accessed directly from the alcohol with a Johnson orthoester Claisen rearrangement or by an ester enolate Claisen rearrangement, respectively.[5hi]

The development and elaboration of the Baylis-Hillman reaction provided a key intermediate for the synthesis of intergerrinecic acid in 87% overall yield. Other reported syntheses are either low yielding and/or multistep.[3a] The Baylis-Hillman reaction has provided a possible synthon for the general synthesis of necic acids.

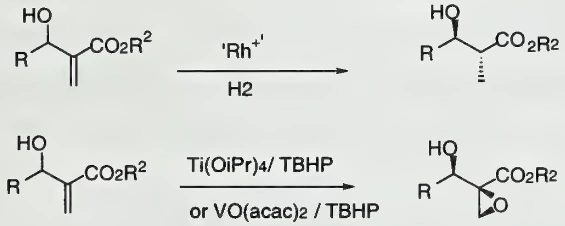
SCHEME IV



Diastereoselective manipulations

The Baylis-Hillman adduct possesses the reactivity of an unsaturated ester with the possibility of stereocontrol by the allylic alcohol. Excellent diastereoselectivities have been achieved for reductions,[6] epoxidations,[7] dihydroxylations,[8] and modest selectivities for radical addition reactions,[10] Michael reactions[xxxii] and [3+2] dipolar cycloadditions.[9] A few of which are highlighted in Scheme V.

SCHEME V



Reduction of the of α-(hydroxyalkyl)acrylates has been achieved with high selectivity[6] (Scheme V). The products bear two asymmetric centers and are formal products of an anti ester enolate aldol. This procedure obviates the traditional need for a bulky ester and sterically demanding aldehyde.

Diastereoselective epoxidations have been have exploited by Markó and Ollis for the potential synthesis of the side chains of Clerodane diterpenes. A survey of epoxidizing agents revealed that remarkably the Sharpless titanium catalyzed epoxidation conditions as well as the VO(acac)$_2$/ TBHP conditions not only produce the syn epoxy alcohol in 76-78% yield, but also with greater than 99:1 selectivity (Scheme VII). The use of basic hydroperoxide protocol provided the only mildly selective (3:2) for the anti diastereomer.[7]

DIASTEREOSELECTIVE BAYLIS-HILLMAN REACTION

The Baylis-Hillman reaction has many opportunities for asymmetric modification The use of chiral tertiary amines, external stereocontrol, is by far the most attractive method, since this would lead to a catalytic enantioselective reaction. Diastereoselective possibilities for internal stereocontrol are chiral acrylates, reaction with chiral metal complexed aldehydes, or α-chiral aldehydes. All of these possibilities have been addressed with varying degrees of success. A few resolutions of the Baylis-Hillman adducts have also been reported.[xxxiii]

External Stereocontrol

Initial reports of the use of tertiary amine alkaloids as catalyst for the reaction acrylonitrile or acrylic esters with aldehydes at atmospheric and high pressure gave enantiomer excesses which ranged from total nonselective to 17% ee. The yields were also poor.[1,11a] These early reports led to the assumption that the quaternary ammonium moiety was too remote for effective stereochemical translation. However, in a very recent communication, Hirama reports enantioselectivities up to 47% using a synthetic chiral DABCO derivative of type **8** (Figure 1) under high pressure.[11b] While this is promising there still is too little information on the mechanism which is critical for ligand design.

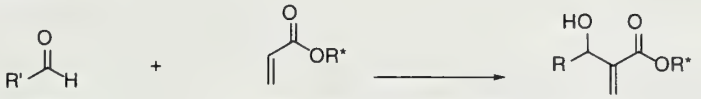
Internal Stereocontrol

Reactions of chiral α-alkoxy(or amino)aldehydes with acrylates have met limited success. The selectivity is only 2-3:1 in favor of the anti product arising from the addition predicted by the Felkin-Anh paradigm.[15c, 11a, xxxiv] An alternative approach to controlling the hydroxyl configuration in the Baylis-Hillman reaction has been demonstrated by Kundig. Enantiomerically enriched ortho substituted benzaldehyde $Cr(CO)_3$ complexes are very effective at generating the coupled product in 90% yield and >98% ee after decomplexation.[18]

Baylis-Hillman reaction with chiral acrylates have obtained products with modest diastereoselectivities.[11a,12] High diastereoselectivities are obtained using high pressure, but yields are lower (Table I). For example, the phenylmenthyl acrylate produces the coupled product in 82% yield and 35% de at atmospheric pressure.[12c] Yet, at 8 Kbar the yield drops to 31% and de increases to 86%.[11a] Likewise, the menthyl acrylate produces the coupled product in 93% yield and only 22% de at atmospheric pressure, but at 7.5 kbar the yield is only 42% of 100% de. When acetaldehyde is employed as the electrophile, there is a definite high pressure effect on yield (95% yield at 0.001 kbar versus 36% yield at 7 kbar), but the diastereoselectivities are both low.[11a] In this case the methyl group is likely too small even under high pressure to significantly affect diastereoselection, but the pressure effect on yield is real. An obvious pressure effect on both yield and diastereoselection exists in these cases; a systematic study of the effect would be very informative. The pressure effect on yield is a reflection of the sensitivity of the equilibrium constant to pressure, similar to the effect that temperature has on equilibrium.[21] The pressure

effects on diastereoselectivity are not without precedents, although not nearly to the same extent that is present in the Baylis-Hillman reaction.[xxxv]

TABLE I The effect of high pressure on yield and diastereoselectivity in reactions of chiral acrylates with aldehydes catalyzed by DABCO.



R*	R'	Kbar	yield (%)	de (%)
Phenylmenthyl	Ph	0.001	82	35
Phenylmenthyl	Ph	8.0	31	86
menthyl	Ph	.001	93	22
menthyl	Ph	7.5	42	100
menthyl	CH_3	0.001	95	14
menthyl	CH_3	7.0	36	16

CONCLUSION

Although the scope of the Baylis-Hillman reaction is generally outlined, the development is still in its infancy. This reaction offers a mild, simple and inexpensive methodology for the construction of substituted acrylates for applications in the synthesis of natural products as well as for access to previously unattainable reactive compounds. Although the efforts to control the newly generated stereocenter have been successful in limited cases, the rational development of an asymmetric catalysts or general chiral auxiliary acrylate is likely, especially if the pressure effect of on diasteroselection is further understood.

REFERENCES

[1]) Drewes, S. E.; Roos, G. H. P. *Tetrahedron* **1988**, *44*, 4653.

[1]) Baylis, A. B.; Hillman M. E. D. German Patent 2,115113, 1972; *Chem. Abstr.* **1972,** *77*, 34174q.

[1]) Drewes, S. E.; Emslie, N. D. *J. Chem. Soc., Perkin Trans. I* **1982,** 2079.

[1]a) Byun, H.; Chandraprakash, R.; Bittman, R. *Tetrahedron Lettt.* **1994**, *35*, 1371. b) Hoffman, H. M. R.; Rabe, J. *Angew. Chem. Int. Ed. Eng.* **1985**, *264,* 94.

[1]a) Ameer, F.; Drewes, S. E.; Emslie, N. D.; Kaye, P. T.; Mann, R. L. *J. Chem. Soc. Perkin Trans. I.* **1983**, 2293. b) Beltaîef, I.; Amri, H. *Synth. Commun.* **1994**, *24*, 2003. c) Hoffman, H. M. R.; Rabe, J. *J. Org. Chem.* **1985**, *50*, 3859. d) van Heerden, F. R.; Huyser, J. J.; Holzapfel, C. W.; *Synth. Commun.* **1994**, *24*, 2863. e) Basavaiah, D.; Bhavani, A. K. D.; Pandiaraju, S.; Sarma, P. K. S. *Synlett* **1995**, 243. f) Basavaiah, D.; Sarma, P. K.; S. Bhavani, A. K. D. *J. Chem. Soc., Chem. Commun.* **1994**, 1091. g) Basavaiah, D.; Sarma, P. K. S. *J. Chem. Soc., Chem. Commun.* **1992**, 955. h) Basavaiah, D.; Pandiaraju, S. *Tetrahedron Lett.* **1995**, 36, 757. i) Mason, P. H.; Emslie, N. D.; Drewes, S. E. *Synth. Commun.* **1995**, *25*, 183. j) Ameer, F.; Drewes, S. E.; Kaye, P. T.; Loizou, G.; Malissar, D. G.; Roos, G. H. P. *S. Afr. J. Chem.* **1987**, *40*, 35. k) Bucholz, R.; Hoffman, H. M. *Helv. Chim. Acta.***1991**, *74*, 1213.

[1]a) Brown, J. M.; Evans, P. L.; James, A. P. *Org. Syn.* **1989**, *68*, 64. b) Brown, J. M. *Angew. Chem. Int. Ed. Eng.* **1987**, *26,* 190. c) Brown, J. M.; Cutting, I.; James, A.P. *Bull. Chim. Soc. Fr.* **1988**, 211. d) Brown, J. M.; James, A. P. *J. Chem. Soc., Chem. Commun.* **1987**, 181. e) Brown, J. M.; James, A. P.; Prior, L. M. *Tetrahedron Lett.* **1987**, *28*, 2179. f) Brown, J. M.; Cutting, I.; Evans, P. L.; Maddox, P. J. *Tetrahedron Lett.* **1986**, *27*, 3307. g) Brown, J. M.; Cutting, I. *J. Chem. Soc., Chem. Commun.* **1985**, 578. h) Ando, D.; Bevan, C.; Brown, J. M.;

Price, D. W. *J. Chem. Soc., Chem. Commun.* **1992**, 592.

[1]a) Bailey, M.; Staton, I.; Ashton, P. R.; Markó, I. E.; Ollis, W. D. *Tetrahedron Asymmetry* **1991**, *2*, 495. b) Bailey, M.; Markó, I. E.; Ollis, W. D. *Tetrahedron Lett.* **1991**, *32*, 2687. c) Bailey, M.; Markó, I. E.; Ollis, W. D.; Rasmussen, P. R. *Tetrahedron Lett.* **1990**, *31*, 4509.

[1]) Markó, I. E.; Giles, P. R.; Janousek, Z.; Hindley, N. J.; Declerq, J.; Tinant, B.; Feneau-Dupont, J.; Svendsen, J. S. *Recueil Trav. Chim.* **1995**, *114*, 239.

[1]a) Annunziata, R.; Benaglia, M.; Cinquini, M.; Cozzi, F.; Raimondi, L. *J. Org. Chem.* **1995**, *60*, 4697. b) Kanemasa, S.; Kobayashi, S. *Bull. Chem. Soc. Jpn.* **1993**, 66, 2685.

[1]a) Giese, B.; Bulliard, M.; Dickhaut, J.; Halbach, R.; Hassler, C.; Hoffman, U.; Hizen, B.; Seen, M. *Synlettt* **1995**, 116. b) Jenn, T.; Heissler, D. *Tetrahedron Lett.* **1992**, *33*, 7227. c) Heissler, D.; Jenn, T.; Nagano, H. *Tetrahedron Lett.* **1991**, *32*, 7587. d) Eastwood, F. W.; Misfsud, R. D.; Perlmutter, P *Aust. J. Chem.* **1994**, *47*, 2187.

[1]a) Gilbert, A.; Heritage, T. W.; Isaccs, N. S. *Tetrahedron Asymmetry* **1991**, *2*, 969. b) Oishi, T.; Oguri, H.; Hirama, M. *Tetrahedron Asymmetry* **1995**, *6*, 1241.

[1]a) Basavaiah, D.; Gowriswari, V. V. L.; Sarma, P. K. S.; Roa, P. D. *Tetrahedron Lett.* **1990**, *31*, 1621. b) Jensen, K.; Roos, G. H. P. *S. Afr. J. Chem.* **1992** *45*, 112. c) Drewes, S. E.; Emslie, N. D.; Khan, A. A. *Synth. Commun.* **1993**, *23*, 1215.

[1]a) Bode, M. L.; Kaye, P. T. *Tetrahedron Lett.* **1991**, *32*, 5611. b) Hill, J. S.; Isaacs, N. S. *J. Phys. Org. Chem.* **1990**, *3*, 285. c) Hill, J. S.; Isaacs, N. S. *J. Chem. Research (s)* **1988**, 330. d) Hill, J. S.; Isaacs, N. S. *Tetrahedron Lett*t. **1986**, *27*, 5007.

[1]) van Rozendaal, E. L. M.; Voos, B. M. W.; Scheeren, H. W. *Tetrahedron* **1993**, *49*, 6931.

[1]a) Roth, F.; Gygax, P.; Frater, G. *Tetrahedron Lett.* **1992**, *33*, 1045. b) Fort, Y.; Berthe, C.; Caubere, P. *Tetrahedron* **1992**, *48*, 6371. c) Manickum, T.; Roos, G. H. P. *S. Afr. J. Chem.* **1994**, *47*, 1.

[1]) Drewes, S. E.; Freese, S. D.; Emslie, N. D.; Roos, G. H. P. *Synth. Commun.* **1988**, *18,* 1565.

[1]) reference 14, Auge, J.; Lubin, N.; Lubineau, A.; *Tetrahedron Lett.*. **1994**, *35*, 7947.

[1]a) Kundig, E. P.; Xu, L. H.; Romanens, P.; Bernardinelli, G. *Tetrahedron Lett.* **1993**, *34,* 7049. b) Kundig, E. P.; Xu, L. H.; Schnell, B. *Synlett* **1994**, 413.

[1]) Kundu, M. K.; Mukherjee, S. B.; Balu, N.; Padmakumar, R.; Bhat, S. V. *Synlett* **1994**, 444.

[1]) Roos, G. H. P.; Rampersadh, P. *Synth. Commun.* **1993**, *23*, 1261.

[1]) see references 13b, 13c, 13d. For a good review of volumes of reaction and activation see van Eldik, R.; Asano, T.; Le Noble, W. J. *Chem. Rev.* **1989**, 549.

[1]) Tsuboi, S.; Takatsuka, S.; Utaka, M. *Chem. Lett.* **1988**, 2003.

[1]) Weichert, A.; Hoffman, H. M. R. *J. Org. Chem.* **1991**, *56*, 4098.

[1]) Hwu, J. R.; Hakimelahi, G. H.; Chou, C. *Tetrahedron Lettt.* **1992**, *33*, 6469.

[1]a) Basavaiah, D.; Bharathi, T. K.; Gowriswari, V. V. L. *Tetrahedron Lett.* **1987**, *28*, 4351. b) Grundke, C.; Hoffmann, H. M. R. *Chem. Ber.* **1987**, *120*, 1461. c) Golubev, A. S.; Galakhov, M. V.; Kolomiets, A. F.; Fokin, A. V. *Bull. Russ. Acad.* **1992**, **41,** 2193. Russian translation *Izv. Akad. Nauk SSSR, Ser. Khim.* **1992**, *12*, 2763.

[1]) Basavaiah, D.; Gowriswari, V. V. L. *Synth. Commun.* **1989**, *19*, 2461.

[1]a) Perlmutter, P.; Teo, C. *Tetrahedron Lett.* **1984**, *25*, 5951. b) Bertenshaw, S.; Kahn, M. *Tetrahedron Lett.* **1989**, *30,* 2731.

[1]a) Drewes, S. E.; Emslie, N. D.; Khan, A. A.; Roos, G. H. P. *Syn. Commun.* **1989**, *19*, 959. b) Janecki, T. *Synth. Commun.* **1993**, *23*, 641. c) van Heerden, F. R.; Huyser, J. J.; Holzapfel, C. W. *Synth. Commun.* **1994**, *24*, 2863.

[1] a) Amri, H.; Villieras, J. *Tetrahedron Lett.* **1986**, *27*, 4307. b) Drewes, S. E.; Emslie, N. D.; Karodia, N. *Synth. Commun.* **1990**, *20*, 1915. c) Basavaiah, D.; Gowriswari, V. V. L.; Bharathi, T. K. *Tetrahedron Lett.* **1987**, *28*, 4591.

[1]) Morita, K.; Suzuki, Z.; Hirose, H. *Bull. Chem. Soc. Jpn.* **1968**, *29*, 3117.

[1]) Villieras, J.; Rambaud, M. *Org. Syn.* **1988**, *66*, 220.

[1]) Pulmutter, P.; Tabone, M. *Tetrahedron Lett.* **1988**, *29*, 949.

[1]a) Burgess, K.; Jennings, L. D. *J. Org. Chem.* **1990**, *55*, 1138. b) Basavaiah, D.; Roa, P. D. *Synth. Commun.* **1994**, *24*, 917.

[1]a) Manickum, T.; Roos, G. H. P. *Synth. Commun.* **1991**, *21*, 2269. b)Drewes, S. E.; Khan, A. A.; Rowland, K. *Synth. Commun.* **1993**, *23*, 183.

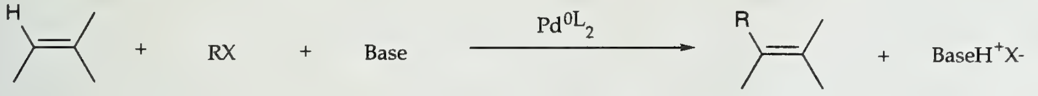
[1]) Jurczak, J.; Tomasz, B. *Tetrahedron* **1986**, 42, 5045.

THE ASYMMETRIC HECK REACTION

Reported by Zhicai Wu September 21, 1995

INTRODUCTION

In the early 1970's, Mizoroki[1]and Heck[2] independently discovered the Palladium(0) catalyzed coupling reaction of an aryl or vinyl halide with an alkene. This methodology, known as the Heck reaction (eq. 1), is a powerful tool for C-C bond formation.[3] However, its use in formation of stereogenic centers was rare until Shibasaki[4] and Overman[5] reported an asymmetric Heck reaction in 1989. Since then, much effort has been dedicated to elucidate the mechanism and improve the stereoselectivity of this type of reaction. This review presents the catalytic intra- and intermolecular asymmetric Heck reaction with regards to its mechanism and applications in enantioselective syntheses of various bioactive molecules.



(eq. 1)

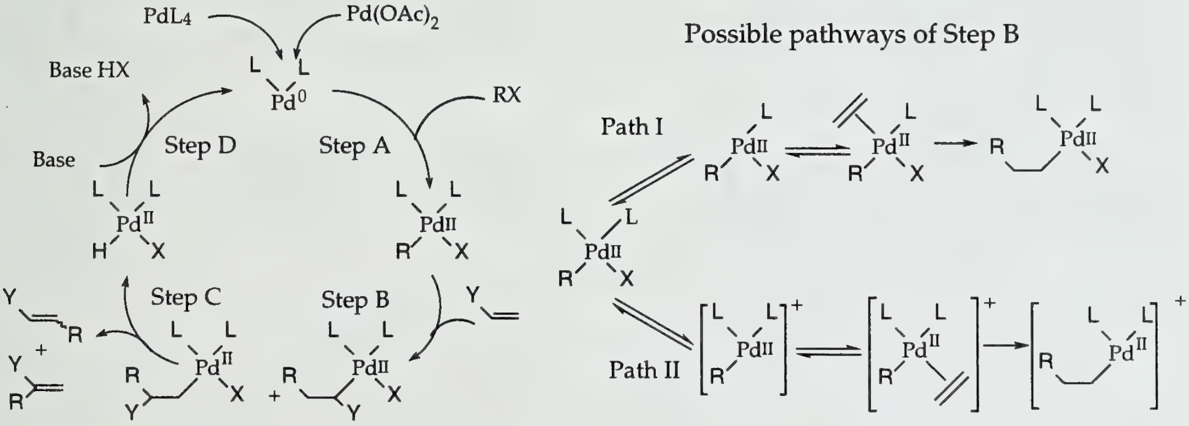
R=aryl, heterocyclic, benzyl, vinyl X=bromide, iodide, triflate L=a ligand for Pd

BACKGROUND

Basic Mechanism

The general accepted mechanism for the Heck reaction involves catalyst formation and a catalytic cycle (Scheme I).[3,6] The catalytically active species is thought to be a coordinatively unsaturated 14-electron palladium(0) complex, which is usually generated *in situ* either directly from tetrakis(triphenylphosphine)palladium(0) or by the reduction of palladium(II) salts in the presence of phosphine ligands. The catalytic cycle consists of four steps: (A) oxidative addition, (B) coordination-insertion, (C) β-hydride elimination-dissociation, (D) catalyst reduction. Among them, coordination-insertion and elimination processes have been shown to occur in a syn manner.

Scheme I



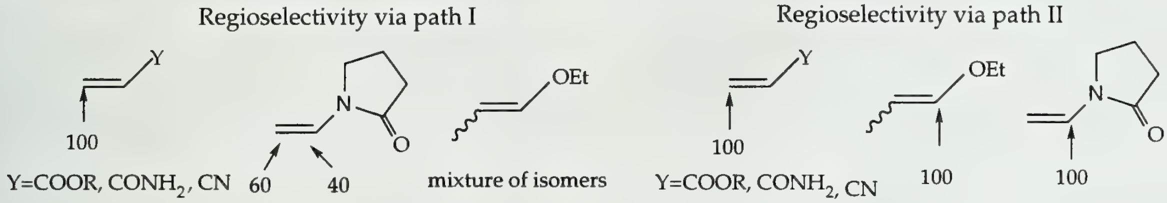
Copyright © 1995 by Zhicai Wu

For Step (B), there are two possible reaction pathways (Scheme I). Path I involves coordination of the olefin via dissociation of one neutral ligand to form a neutral Pd(II) complex. Path II, however, involves coordination of the olefin via dissociation of the anionic ligand to afford the cationic Pd(II) species. These species are responsible for the regioselectivity and enantioselectivity of the Heck reaction.

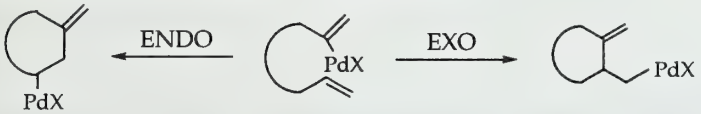
Regiochemistry

For intermolecular Heck reactions (Scheme I & II),[3,6,8] the less substituted site of the olefin is prefered under the usual conditions. Path I of step B is followed and steric effects dominate. However, in the presence of Ag(I) or Tl(I) salts or by the use of triflates instead of halides,[7] path II is followed and electronic effects dominate. The opposite regioselectivity can be achieved for electron rich olefins.

Scheme II

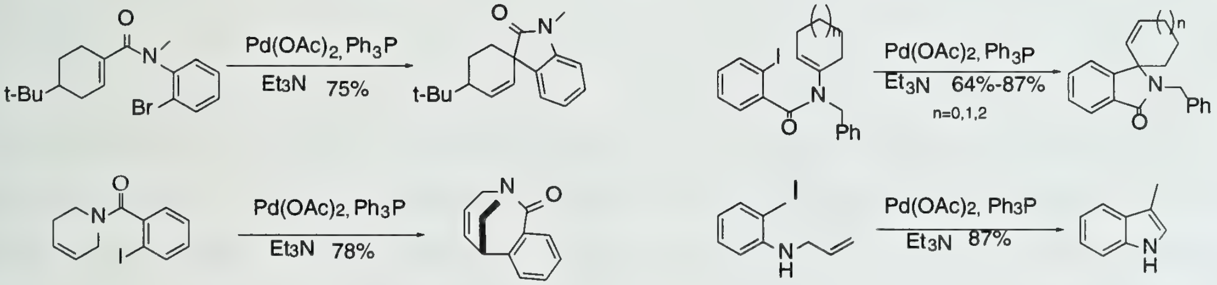


For the intramolecular Heck reaction, when the endo- and exo-trig closures compete (eq. 2), the exo-trig cyclization mode is usually favored (Scheme III).[9] Even when the product ostensibly derived from an endo process has been observed, evidence supporting an initial exo addition followed by rearrangement has been provided (Scheme IV).[9a,10] However, several examples which follow the endo mode have been reported in cases where the exo pathway has been precluded.[9c,11]

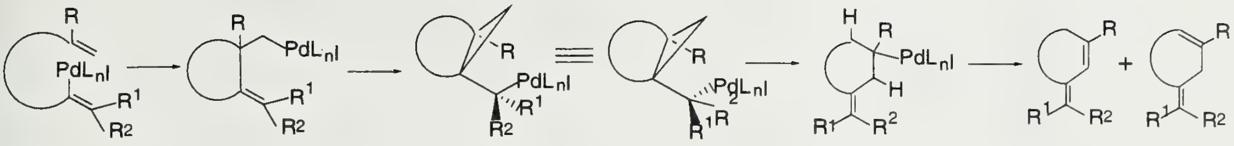


(eq. 2)

Scheme III

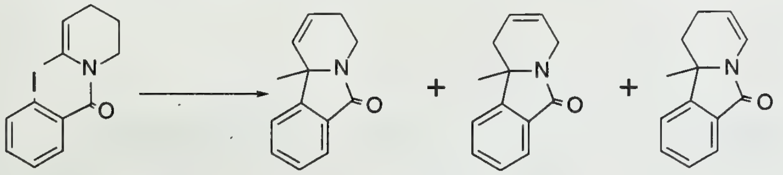


Scheme IV



Alkene Composition of products

A potential problem in Heck reactions is the frequently low selectivity with regard to the position of the double bond in the product. Two processes determine the alkene composition. Process (I) is β-hydride elimination which can proceed in different directions to give the initial isomeric composition of the product.. Process (II) is the readdition of HPdX and subsequent β-hydride elimination to further isomerize the double bond. It was shown by Grigg[9c,12a] and Hallberg[12b] that isomerization in process (II) can be controlled by lowering the reaction temperature[13] or by using Ag(I) or Tl(I) salts (eq. 3). Because of process (I) asymmetric Heck reactions are more or less restricted to cyclic olefins and to the reactions in which quaternary carbon centers are formed, where only one direction of elimination is allowed.



(eq. 3)

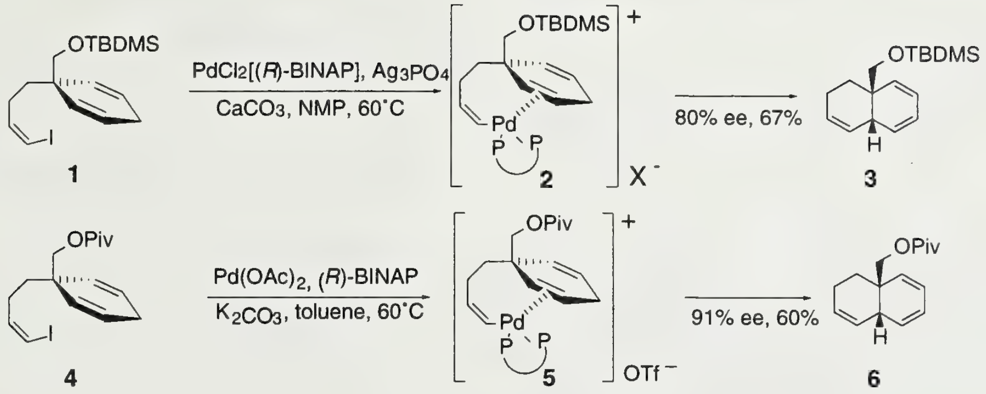
Traditional Conditions: 10% $Pd(OAc)_2$, 20% PPh_3, 100 °C, 1.45:1.27:1 (92%)
Jeffery's Conditions: 10% $Pd(OAc)_2$, 20% PPh_3, Et_4NCl, 30 °C, 2.13:1:0 (72%)
With Tl (I) or Ag(I): 10% $Pd(OAc)_2$, 20% PPh_3, 80 °C 1:0:0 (65%)

ASYMMETRIC REACTION

Intramolecular Formation of Tertiary Carbon Centers using Cyclic Olefins

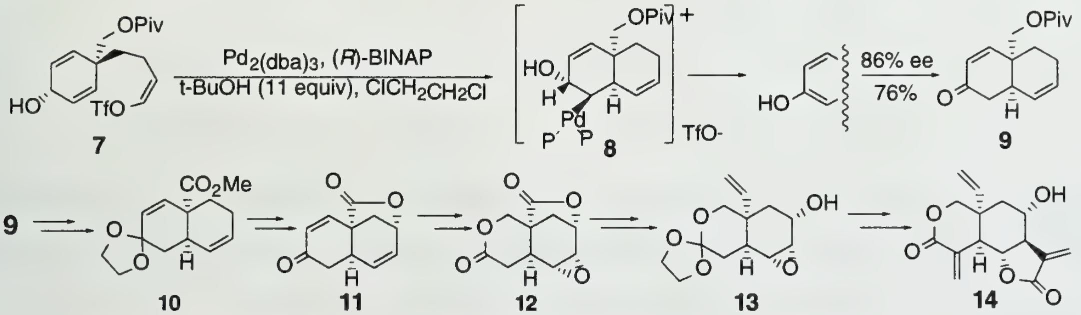
Heck reactions aren't easily amenable to asymmetric synthesis because the stereogenic center generated by olefin insertion may be lost at the subsequent β-H elimination. In the first example[4] of the asymmetric Heck reaction by Shibasaki (Scheme V), a tertiary carbon center was enantioselectively produced using the chiral phosphine ligand BINAP via alkenylation of a cyclic olefin. The hydrogen of this center was not eliminated since it was anti to palladium. After optimization of the catalyst system, the cis-decalin derivative **3** could be synthesized in up to 80% ee from the prochiral alkenyl iodide **1**. It was found that the pre-formed BINAP coordinated catalyst and the presence of Ag(I) salt are necessary to achieve high enantioselectivity. These results indicate the importance of the formation of the cationic intermediate **2** via path II and the efficient coordination of the chiral ligand to Pd. Not surprisingly, the corresponding alkenyl triflate **4,** which can also generate the analogous cationic intermediate **5**, affords **6** with excellent asymmetric induction and in good yield (Scheme V).[15]

Scheme V



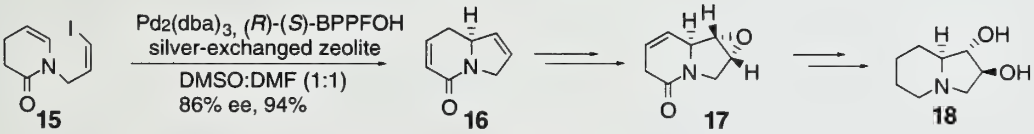
To demonstrate the efficiency of this type of asymmetric Heck reaction for the generation of highly functionalized chiral building blocks, a more functionalized decalin derivative **9,** was prepared in up to 86% ee (Scheme VI).[14b, 15b, 16] Enone **9** was used to prepare several bioactive molecules including the first asymmetric total synthesis of (+)-Vernolepin **14** (Scheme VII).[14b, 15b, 16]

Scheme VI



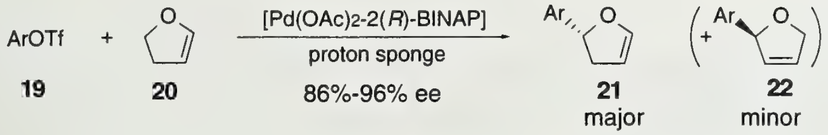
Furthermore, this reaction has also provided a very convenient route to the synthesis of the functionalized indolizidine derivative, **16,** which can be easily transformed to other indolizidine alkaloids such as Lengtiginosin, **18** (Scheme VII).[14b,17]

Scheme VII



Intermolecular Formation of Tertiary Carbon Centers using Cyclic Olefins

In 1991, Hayashi reported the first example of an asymmetric intermolecular Heck reaction: arylation of 2,3-dihydrofuran.[7b,18] A sterically encumbered strong base was found to be important for controlling the enantiomeric excess. The reaction proceeds with excellent enantioselectivity using various aryl triflates with proton sponge as the base(eq. 4).



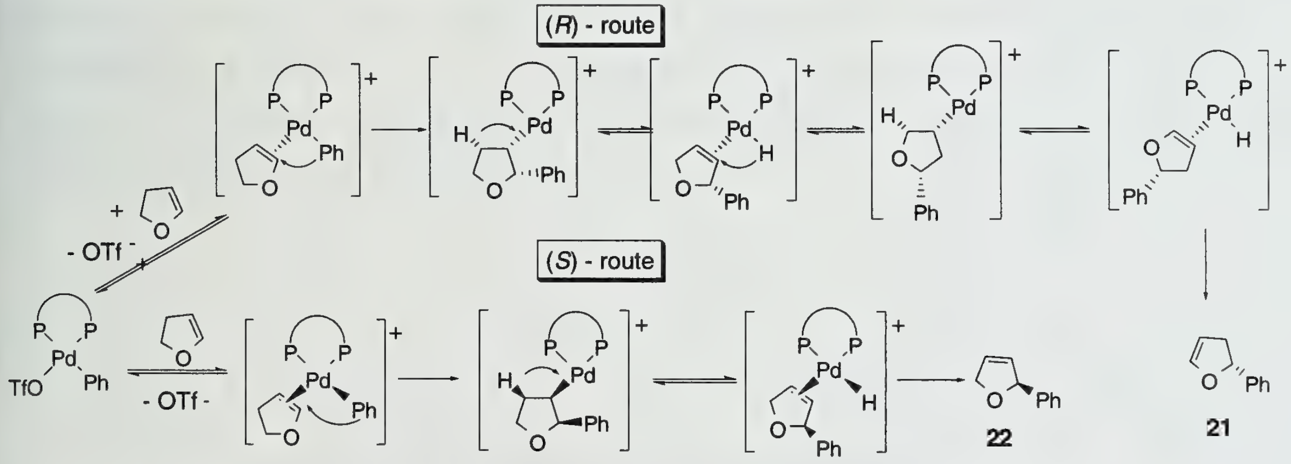
(eq. 4)

The facial selectivity can be explained as shown in Scheme VIII. There are two possible modes for coordination of 2,3-dihydrofuran to the 16 electron cationic species, **23**. Using (*R*)-BINAP, mode (a) is prefered relative to mode (b) because of steric effects. Consequently, the product with the *R* configuration is produced.

Scheme VIII

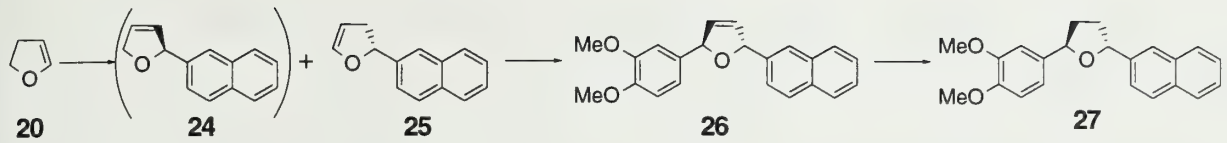
An interesting feature of this reaction is that the two arylation products, **21** and **22**, have opposite configurations. The enantiomeric purity of the major product **21** increases as the ratio of **21/22** decreases. A kinetic resolution process is proposed to account for this observation (Scheme IX).

Scheme IX



This Heck arylation reaction was used to efficiently prepare optically pure **27**, an antagonist of platelet activating factors (Scheme X).

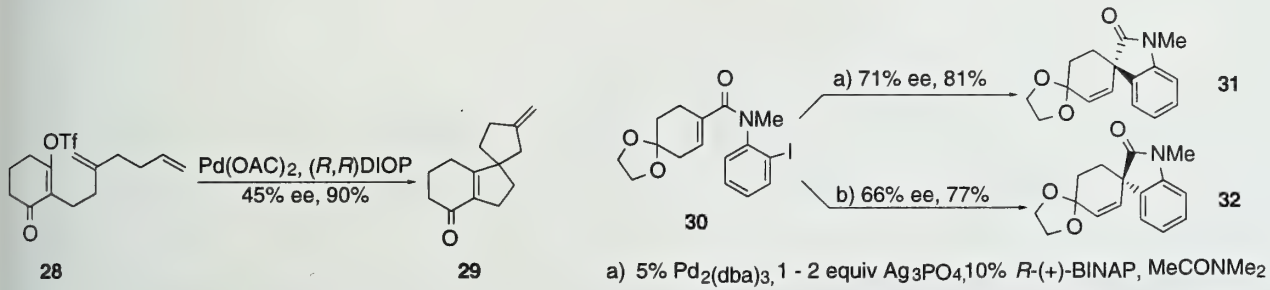
Scheme X



Construction of A Quaternary Carbon Center

In Overman's first example of the creation of a quaternary carbon center, 45% ee was achieved (Scheme XI).[5] It was shown clearly that the reluctance of substituted alkenes to participate in Heck reactions can be overcome and thus congested carbon centers can be forged readily. Further investigation found that either enantiomer of spirooxindoles and related spirocycles can be synthesized in very good enantiomeric excess using BINAP (Scheme XI).[19] The silver and amine-promoted cyclizations proceed with opposite enantioselectivity. This remarkable result is contrary to previous studies.

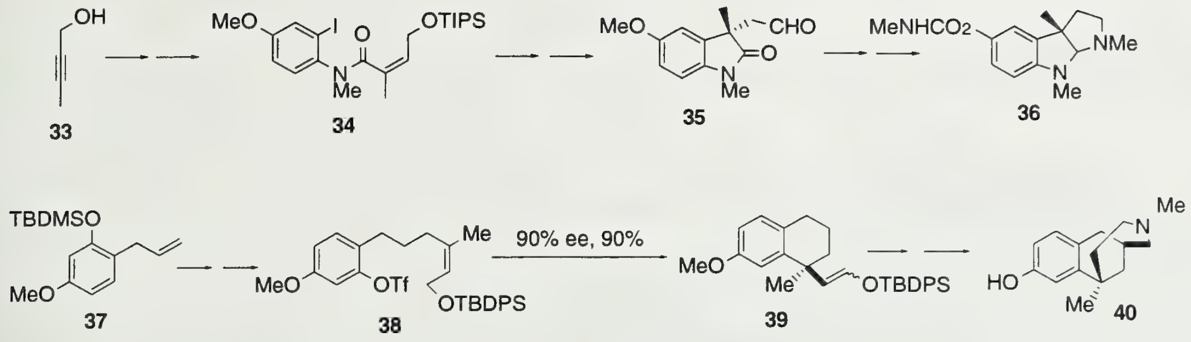
Scheme XI



a) 5% $Pd_2(dba)_3$, 1 - 2 equiv Ag_3PO_4, 10% R-(+)-BINAP, $MeCONMe_2$
b) 10% $Pd_2(dba)_3$, 5 equiv PMP, 20% R-(+)-BINAP, $MeCONMe_2$

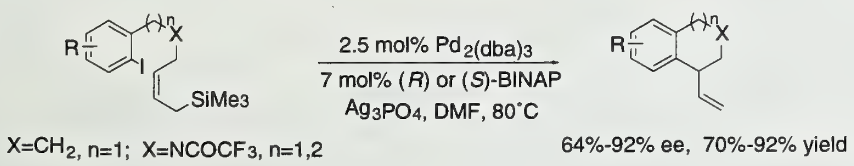
Considering the difficulty of constructing the polycyclic carbon skeletons containing congested quaternary carbon centers, this methodology holds great potential in the area of complex molecule synthesis. Its advantage has been clearly shown by the efficient total synthesis of (-)-physostigmine **36** and (-)-eptazocine **40** (Scheme XII).[19,20] Key steps in both syntheses involve an asymmetric Heck reaction to build a congested quaternary center. The olefin geometry has great effect on enantioselection and only Z-alkenes lead to high enantiomeric excesses.

Scheme XII



Formation of Tertiary Carbon Centers Using Silane -Terminated Alkenes

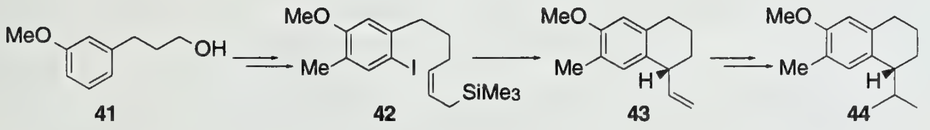
The direction of β-H elimination in the intramolecular Heck reaction can be controlled through the use of silane-terminated alkenes. This expands the scope of asymmetric Heck reaction to include non-cyclic olefins. Formation of stereogenic tertiary carbon centers can be achieved in good to excellent enantioselectivity (eq. 5).[21]



(eq. 5)

To demonstrate the efficiency of this method, a natural norsesquiterpene, 7-desmethyl-2-methoxy calmenene, **44,** was efficiently synthesized using an enantioselective silane-terminated Heck reaction as the key step (Scheme XIII).[22]

Scheme XIII



CONCLUSION

The asymmetric Heck reaction has been developed as a powerful tool for enantioselective formation of C-C bonds and syntheses of various bioactive molecules. Silver(I) salts have played an important role in controlling isomerization as well as achieving regioselectivity and stereoselectivity. More work to understand the contrary results by Shibasaki and Overman along with elucidating the precise mechanism , especially the origin of the enantioselectivity, is desirable. Although this asymmetric reaction has been expanded to include non-cyclic olefins, further work along this line could be worthwile..

REFERENCES

1. Mizoroki, T.; Mori, K.; Ozaki, A. *Bull. Chem. Soc. Jpn.* **1971**, *44*, 581.

2. Heck, R. F.; Nolley, J. P. *J. Org. Chem.* **1972**, *37*, 2320-2322.

3. (a) Heck, R. F. *Acc. Chem. Res.* **1979**, *12*, 146-151. (b) Heck, R. F. *Org. React.* **1982**, *27*, 345-390. (c) Hegedus, L. S. *Tetrahedron*, **1984**, *40*, 2415-2434. (d) Heck, R. F. *Palladium Reagents in Organic Synthesis*; Academic: New York, 1985.

4. Sato, Y.; Sodeoka, M; Shibasaki, M. *J. Org. Chem.* **1989**, *54*, 4738-4739.

5. Carpenter, N. E.; Kucera, D. J.; Overman, L. E. *J. Org. Chem.* **1989**, *54*, 5846-5848.

6. (a) Cabri, W.; Candiani, I. *Acc. Chem. Res.* **1995**, *28*, 2-7. (b) de Meijere, A.; Meyer, F. E. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 2379-2411.

7. (a) Cabri, W.; Candiani, I.; Penco, S. *J. Org. Chem.* **1991**, *56*, 5796-5800. (b) Ozawa, F.; Kubo, A.; Hayashi, T. *J. Am. Chem. Soc.* **1991**, *113*, 1417-1419.

8. Daves, G. D.; Hallberg, A. *Chem. Rev.* **1989**, *89*, 1433-1445.

9. (a) Terpko, M. O.; Heck, R. F. *J. Am. Chem. Soc.* **1979**, 5281-5283. (b) Abelman, M. M.; Oh, T.; Overman, L. E. *J Org. Chem.* **1987**, *52*, 4130-4133. (c) Grigg, R.; Sridharan, V.; Stevenson, P.; Worakun, T. *J. Chem. Soc., Chem. Commun.* **1986**, 1697-1699. (d) Grigg, R.; Stevenson, P.; Worakun, T. *Tetrahedron* **1990**, *46*, 4003-4018. (e) Odle, R.; Blevins, B.; Ratcliff, M.; Hegedus, L. S. *J. Org. Chem.* **1980**, *45*, 2709-2710. (f) Negishi, E.; Zhang, Y.; O'Connor, B. *Tetrahedron Lett.* **1988**, *29*, 2915-2918. (g) Zhang, Y.; O'Connor, B.; Negishi, E. I. *J. Org. Chem.* **1988**, *53*, 5588-5590. (h) Grigg, R.; Stevenson, P.; Worakun, T. *Tetrahedron* **1991**, *47*, 9703-9720. (i) Hegedus, L. S.; Sestrick, M. R.; Harrington, P. J. *J Org. Chem.* **1989**, *54*, 4141-4143. (j) Dankwardt, J. W.; Flippin, L. A. *J. Org. Chem.* **1995**, *60*, 2312-2313.

10. (a) Owczarczyk, Z.; Lamaty, F.; Vawter, E. J.; Negishi, E. *J. Am. Chem. Soc.* **1992**, *114*, 10091-10092. (b) Rawal, V. H.; Michoud, C. *J. Org. Chem.* **1993**, *58*, 5583-5584.

11. Ishibashi, H.; Ito, K.; Hirona, T.; Tabuchi, M.; Ikeda, M. *Tetrahedron* **1991**, *49*, 4173-4180.

12. (a) Grigg, R.; Loganathan, V.; Santhakuma, V.; Sridharan, V. *Tetrahedron Lett.* **1991**, *32*, 687-690. (b) Nisson, K.; Hallberg, A. *J. Org. Chem.* **1990**, *55*, 2464-2470.

13. (a) Jeffery, T. *J. Chem. Soc., Chem. Commun.* **1984**, 1287-1288.

14. (a) Sato, Y.; Sodeoka, M; Shibasaki, M. *Chem. Lett.* **1990**, 1953-1954. (b) Shibasaki, M.; Sodeoka, M. *J. Synth. Org. Chem., Jpn.* **1994**, 956-967. (c) Sato, Y.; Sodeoka, M; Shibasaki, M. *Tetrahedron* **1994**, 50, 371-382.

15. (a)Sato, Y.; Watanabe, S.; Shibasaki, M. *Tetrahedron Lett.* **1992**, *33*, 2589-2592. (b) Ohrai, K.; Kondo, K.; Sokeoka, M.; Schibasaki, M. *J. Am. Chem. Soc.* **1994**, *116*, 11737-11748.

16. Kondo, K.; Sokeoka, M.; Mori, M.; Shibasaki, M. *Tetrahedron Lett.* **1993**, *34*, 4219-4222.

17. Nukui, S.; Sodeoka, M; Shibasaki, M. *Tetrahedron Lett.* **1993**, *34*, 4965-4968.

18. (a) Ozawa, F.; Kubo, A.; Hayashi, T. *Tetrahedron Lett.* **1992**, *33*, 1485-1489. (b) Ozawa, F.; Kubo, A.; Hayashi, T. *Pure & Appl. Chem.* **1992**, *64*, 421-427. (c) Ozawa, F.; Kubo, A.; Hayashi, T. *Organometallics* **1993**, *12*, 4188-4196.

19. (a) Ashimori, A.; Matsuura, T.; Overman, L. E.; Poon, D. *J. Org. Chem.* **1993**, *58*, 6949-6951. (b) Overman, L. E. *Pure & Appl. Chem.* **1994**, *66*, 1423-1430.

20. Takemoto, T.; Sodeoka, M; Shibasaki, M. *J. Am. Chem. Soc.* **1993**, *115*, 8477-8478.

21. Tietze, L. F.; Schimpf, R. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1089-1091.

22. Tietze, L. F.; Raschke, T. *Synlett.* **1995**, 597-598.

MECHANISM OF THE ENANTIOSELECTIVE EPOXIDATION OF UNFUNCTIONALIZED OLEFINS CATALYZED BY (SALEN)MANGANESE(III) COMPLEXES

Reported by Gerald Weisenburger October 2, 1995

INTRODUCTION

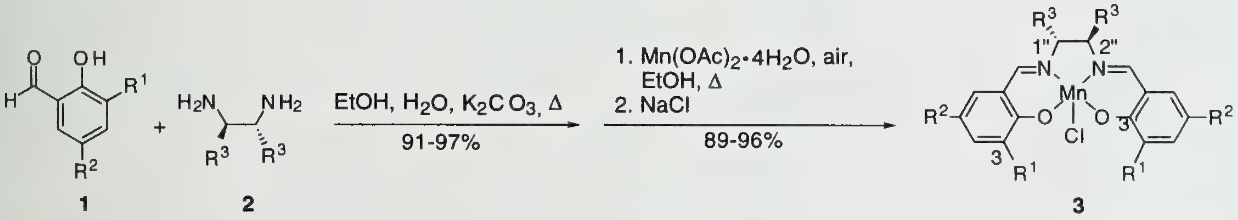
Epoxides serve as important intermediates in synthetic organic chemistry because they can be regio- and stereoselectively converted into a number of useful functional groups.[1] Therefore, an important goal in organic chemistry is the development of a general synthetic method that affords highly enantioenriched epoxides. Enantioselective epoxidation of unfunctionalized olefins may provide the means to accomplish such a goal since the substrate pool is theoretically unlimited.[2] Achiral salen complexes were reported in 1983 to be effective epoxidation catalysts.[3] The development of chiral (salen)Mn(III) complexes as catalysts for the enantioselective epoxidation of unfunctionalized olefins has only recently been reported.[4] Although the method is largely limited to cis-disubstituted olefins, recent reports suggest that the scope may be greatly expanded by ligand modification and control of reaction conditions.[5] A clear understanding of the mechanism is necessary in order to make logical ligand modifications. This review will focus on the mechanism of asymmetric epoxidation catalyzed by (salen)Mn(III) complexes.

BACKGROUND

Preparation of Chiral Salen Complexes

The preparation of salen ligands is accomplished by the condensation of substituted 1,2-diamines, **2**, with two equivalents of a salicylaldehyde derivative, **1**, in refluxing ethanol (Scheme I).[6] Formation of the (salen)Mn(III) complex, **3**, is accomplished by bubbling air through an ethanolic solution of the salen ligand and three equivalents of $Mn(OAc)_2{\bullet}4H_2O$ heated at reflux. NaCl is added and the (salen)Mn(III)Cl complex is isolated as a dark brown solid which can be stored indefinitely.

Scheme I



General Reaction Conditions for Epoxidation

Two general reaction conditions have been developed for the asymmetric epoxidation of olefins catalyzed by chiral (salen)Mn(III) complexes. The most practical method consists of a two-phase system employing aqueous buffered commercial bleach as the stoichiometric oxidant and an organic phase made up of substrate, catalyst, solvent, and additive.[7] These reactions are usually complete in a few hours at room temperature or at 0 °C. A single phase system utilizing iodosylarenes as the stoichiometric oxidant has also been developed. The cost of the oxidant and the formation of aryl iodide by-products make this a less desirable method.[8]

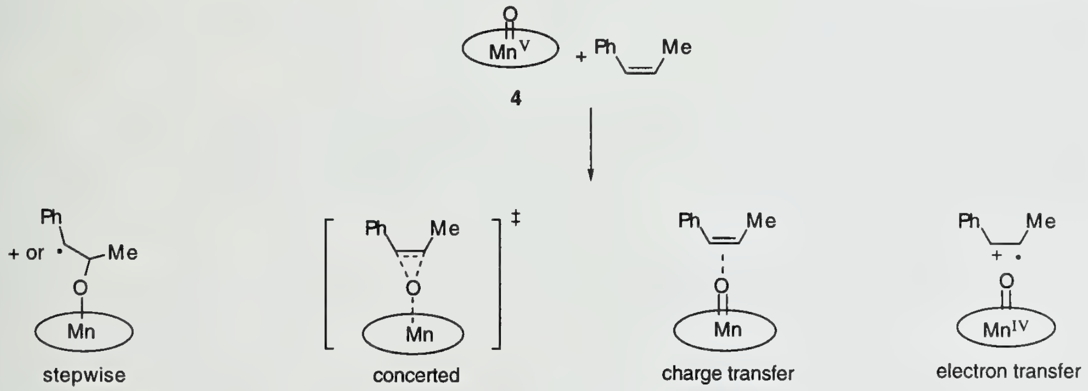
Jacobsen has recently reported an anhydrous, low temperature (-78 °C) protocol utilizing *m*-CPBA as the stoichiometric oxidant and *N*-methylmorpholine-*N*-oxide (NMO) as an additive.[5a] Significant increases in enantiomeric excesses (ee's) with a variety of unfunctionalized olefins have been achieved.

MECHANISM

Proposed Mechanistic Pathways

The first step in the reaction is believed to be oxidation of the (salen)Mn(III) complex to a (salen)Mn(V) oxo complex, **4** (Scheme II).[9] Four mechanistic pathways have been proposed for the reaction between the olefin and the metal-oxo bond: (1) stepwise (radical or cationic), (2) concerted, (3) charge transfer complex formation, and (4) electron transfer.[10] Both the stepwise (radical or cationic) and the concerted mechanisms are reasonable alternatives since they could proceed through highly ordered transition states that would allow for the high level of enantioselectivity observed. Jacobsen argues that the charge transfer and electron transfer mechanisms are unlikely alternatives for stereo-determining steps because they involve only noncovalent interactions in the transition state.[4a] Experimental observations have been used to distinguish between the stepwise mechanism and the concerted mechanism.

Scheme II



Epoxidation Mechanism of Conjugated Olefins

The observation of both cis and trans epoxide formation from pure cis-conjugated olefins strongly suggests that these olefins follow a stepwise mechanism.[11] The starting olefin and product epoxides do not isomerize under the reaction conditions. The substrate influence on cis/trans partitioning in epoxide formation is also consistent with a stepwise mechanism.[12] Substrates with a greater ability to stabilize the intermediate afford higher trans/cis product ratios. This is attributable to longer-lived intermediates which have more time to adopt the trans conformation prior to collapse.

Jacobsen studied a series of substituted methyl cinnamates and found that the log of the cis/trans product ratio correlates well with the σ values of the substituents while correlation with σ^+ values is poor.[12] This data suggests that the intermediate in the proposed stepwise mechanism is radical in nature and not cationic. The lack of significant influence of substituents on the rate also suggests a nonpolar intermediate.

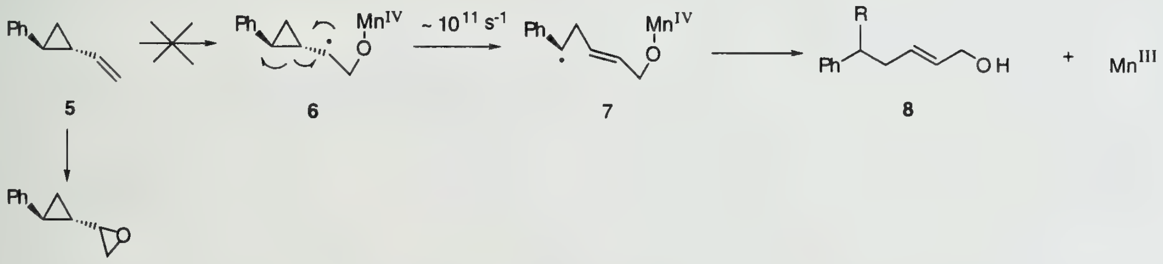
This mechanism can also be used to rationalize the low enantioselectivity observed for terminal conjugated olefins. The competing trans pathway results in a decrease in enantioselectivity because the bond rotation generates enantiomers and not diasteriomers.[13]

Epoxidation Mechanism of Isolated Olefins

Unlike conjugated olefins, isolated olefins do not afford cis and trans diastereomers. There are two possible explanations for this divergence: (1) Collapse of the unstabilized radical intermediate occurs before bond rotation, or (2) the epoxidation of isolated olefins is a concerted process.

A concerted mechanism is consistent with results obtained in the epoxidation of the hypersensitive radical clock, **5** (Scheme III).[10] The rate of rearrangement of the secondary radical, **6**, is estimated[14] to be $10^{11}s^{-1}$ and the rate of epoxide ring closure is estimated to be $2\text{x}10^{10}s^{-1}$.[15] Since the cyclopropyl ring-opened product, **8**, is not observed, the existence of a radical intermediate is highly unlikely.

Scheme III



In general, the epoxidation of isolated olefins affords products with low enantioselectivity, and the epoxidation of conjugated olefins affords products with high enantioselectivity. Jacobsen argues that the stepwise mechanism proposed for conjugated olefins contains an endothermic, irreversible, and stereodetermining first step.[4a] According to the Hammond postulate, the transition state will be intermediate-like, allowing for good stereochemical communication between substrate and catalyst. On the other hand, a concerted process would involve an earlier, more reactant-like transition state, with less interaction between substrate and catalyst. This logic suggests that isolated olefins are best considered as reacting through a concerted mechanism.

THE ORIGIN OF ENANTIOSELECTIVITY

Ligand and Substrate Properties

Certain ligand and substrate properties favor high ee. The most important ligand property is the presence of bulky and/or chiral C3(3') substituents. Substrate properties that favor high ee include (Figure 1) (1) alkynyl, aryl, or alkenyl groups conjugated with the alkene (i vs. ii), (2) a bulky R group as the other vinylic substituent (iv vs. v), (3) a cis olefin geometry (i vs. iii), and (4) the presence of an allylic oxygen substituent (vi vs. vii).

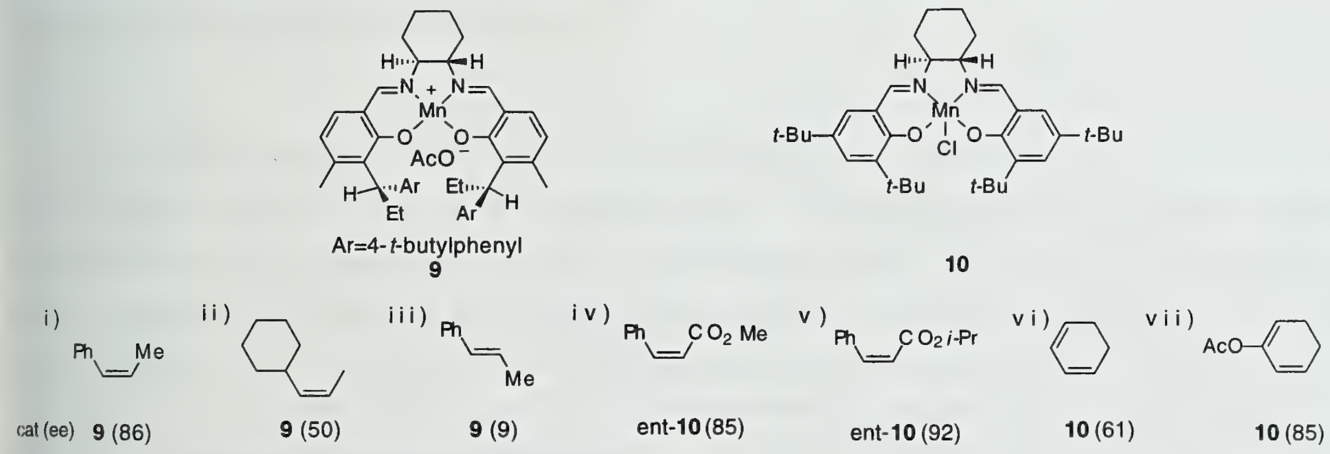
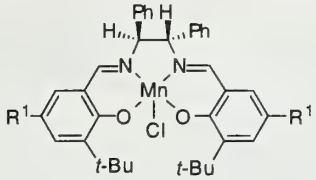


Figure 1. Comparison of substrate properties.

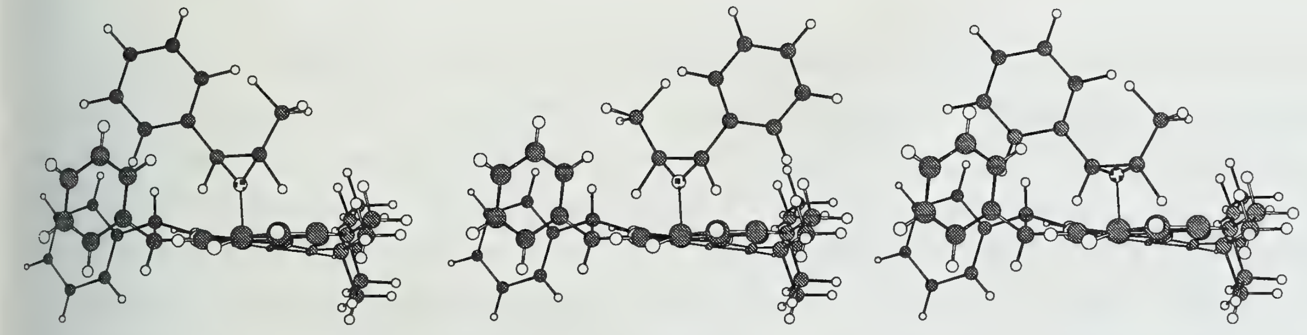
Jacobsen's Models

Jacobsen was the first to report the enantioselective epoxidation of unfunctionalized olefins catalyzed by the chiral salen ligand, **11** (Figure 2).[8] The facial selectivity of the epoxidation was explained by a side-on perpendicular approach of the olefin to the manganese-oxo bond (Figure 2). The hindered

terminus of the olefin is directed away from the *t*-butyl groups, as in **A** and **C**, to avoid the severe steric interaction shown in **B**. Olefin approach from in front of the page, as in **C**, is disfavored by the severe steric interaction between the two phenyl groups. Note that the ligand is C_2 symmetric, so the argument holds for the α- or the β-face.



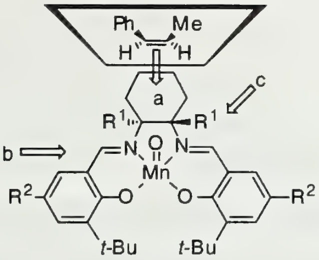
11: R^1=H
12: R^1=*t*-butyl



A (favored)
B
C

Figure 2. Side-on perpendicular approach of the model olefin, *cis*-β-methylstyrene, to the metal oxo bond of **11**. **A** represents the favored approach.

In an effort to improve selectivity, Jacobsen synthesized catalyst **13** (Figure 3) which contains axially locked substituents, R^1, on the diimine bridge.[16] Unfortunately, **13** only provides moderate enantioselectivity. More importantly, the sense of asymmetric induction is opposite to that predicted in Figure 2 (approach b in Figure 3). The change in olefin approach is rationalized by the fact that **13**, which is less hindered than **11** near the diimine bridge, undergoes competitive attack from approach 'a' in Figure 3. Further evidence for this rationale is provided by the observation that **14**, which is even less hindered in the vicinity of the diimine bridge, results in higher ee. Addition of a *tert*-butyl group, as in **15**, results in even higher ee, presumably by strongly disfavoring approach b. Facial selectivity is attributed to the larger substituent on the olefin being directed away from the axial substituent R^1. Both the 1,2-diphenylethylenediamine catalyst, **12**, and the 1,2-diaminocyclohexane catalyst, **15**, afford highly enantioenriched epoxides.



13: R^1=Me, R^2=Me
14: R^1=H, R^2=Me
15: R^1=H, R^2=t-Bu

Figure 3. Approach of olefin to the metal-oxo bond.

Katsuki's Model

Katsuki has reported the epoxidation of olefins with the salen complex, **9**, bearing two additional stereogenic centers on the C3(3') carbons.[17] In a series of papers, he demonstrated that enantiofacial selection of trans olefins is preferentially controlled by the chirality of the C3(3') substituents and that of cis olefins by the chirality of the ethylenediimine bridge.[18] Perhaps the most striking evidence for this conclusion is the epoxidation of *trans*-stilbene and *cis*-*β*-methyl styrene in the presence of **9** and **16** (Figure 4). Both the cis and trans olefins afford moderate ee's when **9** is used as the catalyst. Removal of the chiral diimine bridge, as in **16**, results in a dramatic loss of ee for the cis olefin, but the trans olefin still affords a moderate ee. Katsuki concluded that this observation cannot be rationalized by the side-on perpendicular approach proposed by Jacobsen (approach a, Figure 3) because the trans olefin cannot recognize the chirality of the C3(3') substituents from such an approach. In addition, he pointed out that an oncoming cis olefin following approach 'a' cannot recognize the chirality of the diimine bridge owing to the three axial hydrogen atoms of the cyclohexane ring. Through a series of experiments, Katsuki concluded that the olefin must approach along the nitrogen-manganese bond, (approach c, Figure 3), with the larger olefin substituent oriented away from the bulky C3' substituent.[17]

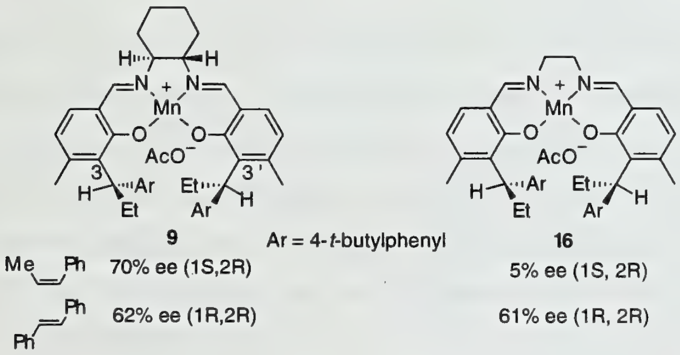


Figure 4. Katsuki's catalysts.

In addition, Katsuki pointed out that enynes approach with the acetylenic substituent oriented toward the less sterically crowded cyclohexane side.[19] The usual steric argument cannot be used to explain this observation because acetylenes are less sterically demanding than alkyl groups. He concluded that olefinic substituents rich in π-electrons are repelled by the π-electron rich salicylaldehyde moiety. On this basis, facial selectivity is dependent on both steric and electronic factors.

The recent reports of high enantioselectivity when trisubstituted[5c,5d] and tetrasubstituted[5b] olefins are used as substrates complicates the debate. Jacobsen reports a reversal in the sense of asymmetric induction compared with cis-disubstituted olefins. He proposes a skewed, side-on approach transition-state model . Katsuki, on the other hand, states that these results are rationalized by taking π-π electronic repulsive forces into consideration. Additional experimental data is needed to help answer this intriguing question.

Enantioselectivity After the First Irreversible Step

As noted earlier, conjugated olefins afford both cis and trans epoxides and a radical stepwise mechanism was proposed to account for this observation. Since olefin isomerization is not observed under the conditions of the reaction, the first step is thought to be irreversible.[20] One would expect that the enantiomeric excesses of the cis and trans epoxides would be identical since the selectivity occurs in the step that leads to the common radical intermediate. However, the experimentally observed ee's of cis and trans epoxides are quite different. This may be explained by the fact that the common intermediate is generated as a mixture of two diastereomers. Therefore, both the degree of cis/trans partitioning in the major and minor transition states and the facial selectivity in the first step determine the ee's of the products. Jacobsen has shown that lowering the reaction temperature (-78 °C) results in enhanced facial selectivity and suppression of the trans pathway.[13] Significant increases in enantioselectivity, especially for terminal olefins, has been reported using this new low temperature methodology.[5a]

CONCLUSION

The enantioselective epoxidation of unfunctionalized olefins catalyzed by (salen)Mn(III) complexes affords highly enantioenriched epoxides. Although the method is largely limited to cis-disubstituted olefins, recent reports of high enantioselectivities achieved with trisubstituted and tetrasubstituted olefins greatly expand the scope of the reaction. Additional experimental data is needed to help elucidate the mode of selectivity so that improved catalysts can be designed through logical ligand modifications. The facile synthesis of the catalyst and its adaptability to large or small scale reactions will facilitate its use in a variety of laboratory and commercial syntheses.[12,21]

REFERENCES

1. Rossiter, B. E. In *Asymmetric Synthesis*; Morrison, J. D., Ed.; Academic: New York, 1986; Vol. 5, p. 193.
2. For reviews see: (a) Kagan, H. B.; Mimoun, H.; Mark, C.; Schurig, V. *Angew. Chem. Int. Ed. Engl.* **1979**, *18*, 485. (b) Davis, F. A.; Chattopadhyay, S. *Tetrahedron Lett.* **1986**, *27*, 5079. (c) Groves, J. T.; Viski, P. *J. Org. Chem.* **1990**, *55*, 3628.
3. Siddall, T. L.; Miyaura, N.; Huffman, J. C.; Kochi, J. K. *J. Chem. Soc., Chem. Commun.* **1983**, 1185.
4. For reviews see: (a) Jacobsen, E.N. In *Catalytic Asymmetric Synthesis*, Ojima, I., Ed.; VCH: New York, 1993; Chapter 4.2. (b) Katsuki, T. *Coordination Chemistry Reviews* **1995**, *140*, 189.
5. (a) Palucki, M.; McCormick, G. J.; Jacobsen, E. N. *Tetrahedron Lett.* **1995**, *36*, 5457. (b) Brandes, B. D.; Jacobsen, E. N. *Tetrahedron Lett.* **1995**, *36*, 5123. (c) Brandes, B. D.; Jacobsen, E. N. *J. Org. Chem.* **1994**, *59*, 4378. (d) Fukuda, T.; Irie, R.; Katsuki, T. *Synlett* **1995**, 197. (e) Chang, S.; Galvin, J. M.; Jacobsen, E. N. *J. Am. Chem. Soc.* **1994,** *116*, 6937.
6. Larrow, J. F.; Jacobsen, E. N.; Gao, Y.; Hong, Y.; Nie, X.; Zepp, C. M. *J. Org. Chem* . **1994**, *59*, 1939.
7. Zhang, W.; Jacobsen, E. N. *J. Org. Chem.* **1991**, *56*, 2296.
8. Zhang, W.; Loebach, J. L.; Wilson, S. R.; Jacobsen, E. N. *J. Am. Chem. Soc.* **1990**, *112*, 2801.
9. Srinivasan, K.; Michaud, P.; Kochi, J. K. *J. Am. Chem. Soc.* **1986**, *108,* 2309.
10. Fu, H.; Look, G. C.; Zhang, W.; Jacobsen, E. N.; Wong, C. -H. *J. Org. Chem.* **1991**, *56*, 6497.
11. Lee, N. H.; Jacobsen, E. N. *Tetrahedron Lett.* **1991**, *32*, 6533.
12. Jacobsen, E. N.; Deng, L.; Furukawa, Y.; Martínez, L. E. *Tetrahedron* **1994,** *50*, 4323.
13. Palucki, M.; Pospisil, P. J.; Zhang, W.; Jacobsen E. N. *J. Am. Chem. Soc.* **1994**, *116,* 9333.
14. He, G.-X.; Bruice, T. C. *J. Am. Chem. Soc.* **1991**, *113*, 2747 and references cited.
15. Bowry, V. W.; Lusztyk, J.; Ingold, K. U. *J. Am. Chem. Soc.* **1989**, *111*, 1927.
16. Jacobsen, E. N.; Zhang, W.; Muci, A. R.; Ecker, J. R., Deng, L. *J. Am. Chem. Soc.* **1991**, *113*, 7063.
17. Hosoya, N.; Hatayama, A.; Yanai, K.; Fujii, H.; Irie, R.; Katsuki, T. *Synlett* **1993**, 641.
18. (a) Irie, R.; Noda, K.; Ito, Y.; Katsuki, T.*Tetrahedron Lett.* **1991**, *32*, 1055. (b) Irie, R.; Noda, K.; Ito, Y.; Matsumoto, N.; Katsuki, T. *Tetrahedron Asymm.* **1991**, *2*, 481. (c) Hosoya, N.; Irie, R.; Ito, Y.; Katsuki, T. *Synlett* **1991**, 691. (d) Sasaki, H.; Irie, R.; Katsuki, T. *Synlett* **1993**, 300.
19. Hamada, T.; Irie, R.; Katsuki, T. *Synlett* **1994**, 479.
20. Zhang, W.; Lee, N. H.; Jacobsen, E. N. *J. Am. Chem. Soc.* **1994**, *116*, 425.
21. For examples see: (a) Chang, S.; Lee, N. H.; Jacobsen, E. N. *J. Org. Chem.* **1993**, *58*, 6939. (b) Lee, N. H.; Muci, A. R.; Jacobsen, E. N. *Tetrahedron Lett.* **1991**, *32*, 5055. (c) Deng, L.; Jacobsen, E. N. *J. Org. Chem.* **1992,** *57*, 4320. (d) Chang, S.; Heid, R. M.; Jacobsen, E. N. *Tetrahedron Lett.* **1994**, *35*, 669. (e) Hamada, T.; Daikai, K.; Irie, R.; Katsuki, T. *Synlett* **1995**, 407. (f) Hatayama, A.; Hosoya, N.; Irie, R.; Ito, Y.; Katsuki, T. *Synlett* **1992**, 407.

ASYMMETRIC ENZYMATIC CATALYSIS WITH CROSS-LINKED ENZYME CRYSTALS (CLECs)

Reported by Bryan Yeung October 5, 1995

INTRODUCTION

Enzymes are unique synthetic reagents that provide for enantioselective reactions in high yield and obviate the need for protecting groups because of their functional group specificity.[1] Despite these advantages, enzymes typically are not viewed as common reagents and, as a result, a standard methodology for their use as catalysts in organic chemistry has been slow to evolve. A common misperception is that all enzyme-related chemistry must occur in aqueous media. Although typical organic compounds are only soluble in non-aqueous solution, the pioneering work of Klibanov[1] and others has demonstrated that enzymes retain at least some of their activity in organic solvents. These studies have shown that pure organic solvent or organic/aqueous binary solutions do in fact promote enzyme-catalyzed transformations.

This abstract will focus on the cross-linked crystals of thermolysin and *Candida rugosa* lipase and their use as viable synthetic organic reagents. These enzymes are presently the only two published examples of CLECs. Thermolysin has been used to stereospecifically synthesize small peptides while *Candida rugosa* lipase resolves chiral esters, acids,[2] amines,[3] and alcohols.[4] Their catalytic activity is maintained in several physical forms: (1) crude enzyme, (2) purified enzyme, (3) enzyme either adsorbed or covalently bound to solid supports and (4) cross-linked enzyme crystals which are covalently linked.

Although the native enzyme might afford a higher initial rate of activity than that of the immobilized enzyme, the overall stability gained from cross-linking allows reactions to

Copyright © 1995 by Bryan Yeung

take place under non-native conditions, aids in catalyst recovery, and prolongs the lifetime of the enzyme complex.

Adapting enzymes into efficient and viable organic synthetic reagents has been met with mixed results. Pure enzymes afford higher enantioselectivities but at increased cost and with lower stability. Immobilization of enzymes, which first must be purified, only moderately increases stability but allows for reuse and ease of recovery. The inert support, in addition to its cost, dilutes enzyme. Cross-linked enzyme crystals have combined the benefits of the pure enzyme with the advantage of immobilized proteins. In principle increased enantioselectivity and stability should result from this technique.

BACKGROUND

Thermolysin

Thermolysin is a metalloprotease (MW = 34.6 kD) isolated from the thermophile *Bacillus thermoproteolyticus*.[5] The active site contains coordinated Zn^{2+} that is required for catalytic activity and four calcium ions which are necessary for thermostability.[6] The native function of thermolysin is degradation of proteins by the cleavage of the C-N amide bond in a polypeptide chain. Peptide bond cleavage in thermolysin is an equilibrium reaction where the reaction lies far towards the hydrolysis products. By altering the reaction conditions, the reversible reaction, i.e. peptide bond formation, can be induced.[7]

Candida rugosa lipase

Candida rugosa lipase (formerly *cylindracea*, MW = 60 kD) is isolated from the fungus of the same name.[8] Lipases promote the hydrolysis of triacylglycerols to glycerol and the corresponding fatty acids.[9] Not surprisingly, *Candida rugosa* lipase also possesses the ability to hydrolyze other carboxylic acid derivatives (Figure 1).

The mechanism of ester hydrolysis is similar to that found in the serine proteases. The active site contains a serine residue that performs a nucleophilic attack upon the

substrate ester carbonyl and forms a tetrahedral intermediate that subsequently releases an alcohol. The acyl-enzyme intermediate complex can then undergo another S_N2 attack by water, which upon formation of another tetrahedral intermediate, goes on to regenerate the enzyme and the corresponding acid.[10]

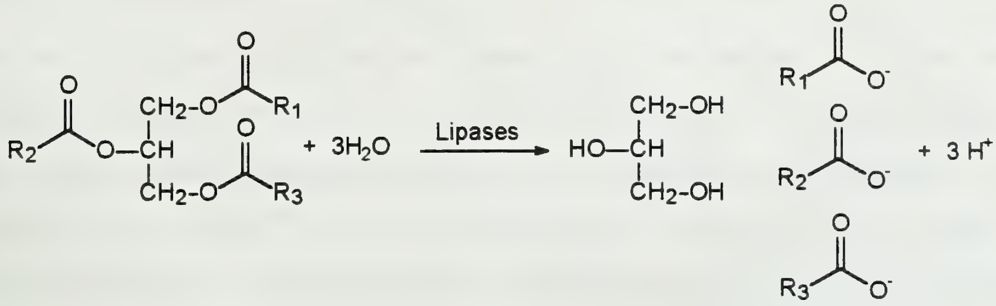


Figure 1. Hydrolysis of triacylglycerols to glycerol and fatty acids.

CROSS-LINKED ENZYME CRYSTALS (CLECs)

CLEC technology cross-links crystallized enzymes using a bifunctional reagent such as glutaraldehyde.[11] This cross-linking is carried out such that crystallinity and activity are maintained.[12] In the crystalline form the enzyme is near its theoretical packing limit, therefore the crystal has the highest concentration of catalyst per unit area. Covalent bonding of the molecules within the crystal makes the catalyst insoluble in aqueous and organic solvent as well as easily recoverable from the reaction media. Thus, cross-linking affords the enzyme stability under conditions typically incompatible with normal enzyme function. CLECs have been used at elevated temperatures, in multiple reaction cycles, in near-anhydrous organic solvents, and in aqueous-organic solvent mixtures.[12]

In 1964 Quiocho and Richards proposed a glutaraldehyde cross-link to improve the stability of enzyme crystals used in diffraction experiments.[13] When cross-linked enzymes were compared with the native form, it was found that the x-ray diffraction pattern of the two were nearly identical. Cross-linked carboxypeptidase-A crystals were shown to maintain some catalytic activity in crystalline state and remained insoluble under

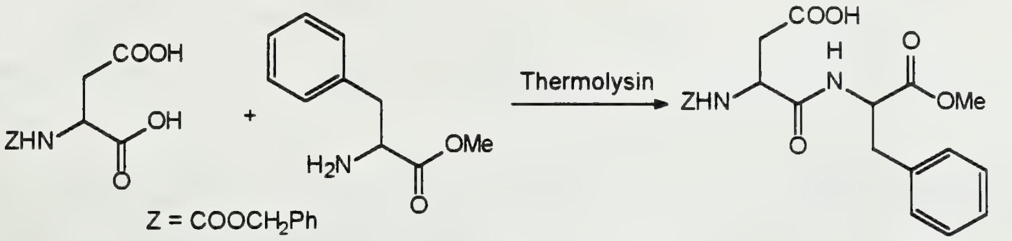
high salt conditions that normally dissolved non-cross-linked enzymes.[14] CLECs of carboxypeptidase-A were never subjected to organic solvents in the above experiments. It was believed that cross-linking modified the enzyme such that the observed reduction of activity was a necessary side-effect. The cross-linking inhibited the enzyme by possibly restricting its movement and reducing pore size. Such crystals might therefore be impervious to organic molecules and unsuitable as biocatalysts. To the contrary, microcrystals of thermolysin-CLECs (around 50 μm in size) are extremely porous and, unlike crystals of small organic molecules, contain large channels (diameter ~ 26 Å) through the crystal matrix.[15] These channels fill with solvent and make up 50% of the crystal's total volume. The large channel size allows small organic molecules to diffuse into the crystal where they can react and subsequently return to the bulk media.[16]

In the crystalline state, protein-protein interactions (primarily electrostatic forces and hydrophobic interactions) increase the enzyme's thermostability relative to its solution state. This added stability increases a protein's ability to resist heat and other denaturants by preventing unfolding, aggregation, or dissociation.[17] In addition, the crystalline form is required for CLEC stability and activity. Both non-crystalline forms of cross-linked enzyme or its precipitate do not show stability beyond that of the native soluble enzyme. In the presence of a high concentration of exogenous proteases, thermolysin-CLECs resist degradation and retain complete activity after four days of incubation[11] whereas soluble thermolysin is digested in 90 minutes.

Peptide Synthesis

Cross-linked crystals of both thermolysin and *Candida rugosa* lipase have been developed. Thermolysin-CLECs have been used in the repetitive batch synthesis of the aspartame dipeptide precursor **3,** (Figure 2) as well as the 31 amino acid residue B-chain of human insulin. The catalyzed coupling is highly stereospecific for L-amino acids and is free of racemization. Both of these syntheses were carried out in the presence of organic solvents. The aspartame precursor was synthesized in ethyl acetate (99% yield) while the oxidized insulin B chain synthesis was carried out in 90% DMF/EtOH (1:1) in a 46% yield.[16] The moderate yield for the insulin B chain synthesis may reflect the synthetic

limitations of thermolysin-CLECs due to chain length or competing reactions with the N-terminus. Immobilized thermolysin has already been examined as an industrial alternative to the present chemical synthesis of aspartame synthesis.[18]



Z-L-Aspartic acid (**1**) DL-Phenylalnine-OMe (**2**) Z-L-Aspartame precursor (**3**)

Figure 2. Thermolysin catalyzed synthesis of aspartame precursor.

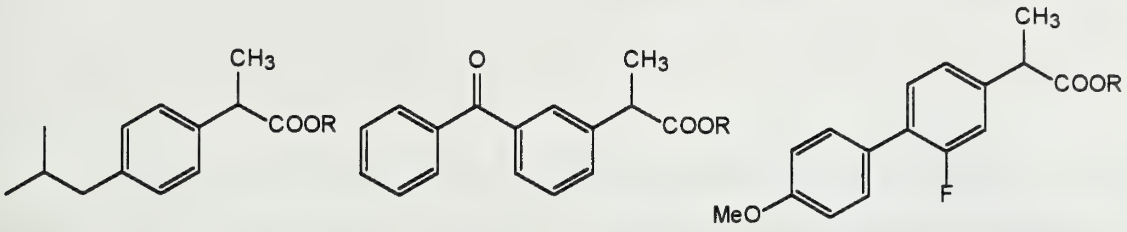
Enantioselectivity

Cross-linked *Candida rugosa* lipase crystals have been used in the resolution of chiral drugs and show increased stereoselectivity relative to the crude enzyme mixture. Several α-substituted carboxylic acids and alcohols have been resolved via ester hydrolysis by both commercial *Candida rugosa* lipase and *Candida rugosa* lipase-CLECs. Because of the high purity of the catalyst afforded by CLECs, a ten-fold increase in the amount of the commercial enzyme relative to CLECs is required for the same amount of conversion.[19]

Numerous hydrolysis experiments with *Candida rugosa* lipase have been carried out to resolve racemic esters.[20] Its hydrolysis preference for one enantiomer over the other has immense commercial value in the resolution of chiral drugs. Since *Candida rugosa* lipase CLECs are more robust catalysts, they may be more amenable to industrial use than are their native forms. Recent experiments have shown that *Candida rugosa* lipase CLECs resolve the hydrolysis products of chiral drugs with higher enantioselectivity than that of the crude enzyme.[19]

Ibuprofen (**4**), Ketoprofen (**5**), and Flurbiprofen (**6**) are nonsteroidal anti-inflammatory drugs that are marketed as racemates (Chart 1), even though it is known that their activity resides solely within the (*S*)-enantiomers.

Chart 1



(4) **a**: R = H
b: R = Me

(5) **a**: R = H
b: R = CH_2CH_2Cl

(6) **a**: R = H
b: R = CH_2CH_2Cl

In the hydrolysis of **4**, **5**, and **6**, *Candida rugosa* lipase-CLECs reacts with the (*S*)-ester stereospecifically to afford the corresponding (*S*)-acid product while the (*R*)-ester remains unreacted. These allows for the efficient resolution of the racemic mixture to exclusively afford the pharmacologically active enantiomer.

Table 1. Comparison of *Candida Rugosa* Lipase CLEC and crude forms

substrate	% e.e.			% conversion (time, h)		
	CLEC	pure	crude	CLEC	pure	crude
4b	94.6 (*S*)-**4a**	95 (*S*)-**4a**	81.8 (*S*)-**4a**	22 (5)	42 (48)	39 (34)
5b	91.1 (*S*)-**5a**	52 (*S*)-**5a**	33.3 (*S*)-**5a**	49 (40)	32 (120)	37 (40)
6b	94.3 (*S*)-**6a**	80 (*S*)-**6a**	61.1 (*S*)-**6a**	34 (48)	14 (72)	34 (16)

For substrate esters **4b-6b**, CLECs have activity and enantioselectivity that is consistently higher than that of the crude enzyme and about equal to or exceeding that of the pure enzyme. CLECs, unlike the crude enzymes, do not contain impurities that compete for the substrate. It is believed that the high enantioselectivity and activity of the CLECs is the result of their purity.

CONCLUSION

The use of enzymes in asymmetric catalysis has been well documented, however previous techniques for optimizing stability and activity have met with only moderate

success. The application of CLECs allow enantioselective reactions in environments that are incompatible with normal enzyme function, thereby has expanding the range of reactions accessible to biocatalysis. Since the catalyst has activity equal to that of pure soluble enzyme, is easily recovered and recyclable. CLECs have potential for large scale industrial applications.

In the future cross-linked enzyme crystals of broader array of enzymes need to be prepared. This will extend the utility of the catalysts and perhaps their acceptance as standard synthetic reagents. Irregardless, it will be some time until enzymes become a part of the normal repertoire of organic chemists.

Industrial applications that presently involve enzyme catalysis utilize the crude form for large scale preparations where high selectivity is not required.[21] Since CLECs combine the activity of a pure enzyme with greater stability and recoverablility than an immobilized one, they are amenable to more complex range of transformations.

REFERENCES

1) (a) Santaniello, E.; Ferraboschi, P.; Grisenti, P.; Manzocchi, A. *Chem. Rev.* **1992**, *92*, 1071. (b) Klibanov, A. M. *Acc. Chem. Res.* **1990**, *23*, 114.

2) (a) Wu, S.-H.; Guo, Z.-W.; Sih, C. J. *J. Am. Chem. Soc.* **1990**, *112*, 1990. (b) Hernaiz, M. J.; Sanchez-Montero, J. M.; Sinisterra, J. V. *Tetrahedron* **1994**, *50*, 10749.

3) Kitaguchi, H.; Fitzpatrick, P. A.; Huber, J. E.; Klibanov, A. M. *J. Am. Chem. Soc.* **1989**, *111*, 3094.

4) King, C.-H.; Margolin, A. L.; *Tetrahedron: Asymmetry* **1993**, *4*, 943.

5) Endo, S. *J. Ferment. Technol.* **1962**, *40*, 346.

6) (a) Latt, S. A.; Holmquist, B.; Vallee, B. L. *Biochem. Biophys. Res. Comm.* **1969**, *37*, 333. (b) Feder, J.; Garrett, L. R.; Wildi, B. S. *Biochemistry* **1971**, *10*, 4552.

7) Halling, P. J. *Enzyme Microb. Technol.* **1984**, *6*, 513.

8) (a) Grochulski, P.; Bouthillier, F.; Kazlauskas, R. J.; Serreqi, A. N.; Schrag, J. D.; Ziomek, E.; Cygler, M. *Biochemistry* **1994**, *33*, 3494. (b) Grochulski, P.; Li, Y.; Schrag, J. D.; Bouthillier, F.; Smith, P.; Harrison, D.; Rubin, B.; Cygler, M. *J. Biol. Chem.* **1993**, *268*, 12443.

9) Stryer, L. *Biochemistry*; W. H. Freeman; New York,1988; p 471.

10) Kester, W. R.; Matthews, B. W. *Biochemistry* **1977**, *6*, 2506

11) Martinek, K.; Torchilin, V. P.; *Meth. Enzymol.* **1988**, *137*, 615.

12) St. Clair, N. L.; Navia, M. A. *J. Am. Chem. Soc.* **1992**, *114*, 7314.

13) Quiocho, F. A.; Richards, F. M. *Proc. Natl. Acad. Sci. U.S.A.* **1964**, *52*, 833.

14) (a) Quiocho, F. A.; Richards, F. M. *Biochemistry* **1967**, *5*, 4062. (b) Quiocho, F. A.; Bishop, W. H.; Richards, F. M. *Proc. Natl. Acad. Sci. U.S.A.* **1967**, *57*, 525.

15) LaLonde, J. J. *Chemica Oggi* **1995**, *13*, 33.

16) Persichetti, R. A.; St. Clair, N. L.; Griffith, J. P.; Navia, M. A.; Margolin, A. L. *J. Am. Chem. Soc.* **1995**, *117*, 2732.

17) Guisan, J. M. *Enzyme Microb. Technol.* **1988**, *10*, 375.

18) (a) Nagayasu, T.; Miyanaga, M.; Tanaka, T.; Sakiyama, T.; Nakanishi, K. *Biotechnol. Bioeng.* **1994**, *43*, 1118. (b) Nakanishi, K.; Takeuchi, A.; Matsuno, R.; *Appl. Microbiol. Biotechnol.* **1990**, *32*, 633-636. (c) Nagayasu, T.; Miyanaga, M.; Tanaka, T.; Sakiyama, T.; Nakanishi, K. *Biotechnol. Bioeng.* **1994**, *43*, 1108.

19) Lalonde, J. J.; Govardhan, C.; Khalaf, N.; Martinez, A. G.; Visuri, K.; Margolin, A. J. *J. Am. Chem. Soc.* **1995**, *117*, 6845.

20) (a)Wu, S.-H.; Guo, Z.-W.; Sih, C. J. . *J. Am. Chem. Soc.* **1990**, *112*, 1990. (b) Colton, I. J.; Ahmed, S. N.; Kazlauskas, R. J. *J. Org. Chem.* **1995**, *60*, 212.

21) Margolin, A. L. *Enzyme Microb. Technol.* **1993**, *15*, 266.

CHROMIUM(0) MEDIATED HIGHER-ORDER CYCLOADDITIONS

Reported by Mark Seierstad October 9, 1995

INTRODUCTION

Eight- and ten-membered rings have always been interesting subjects in organic chemistry; their frequent appearance in natural products make them prime targets for synthetic endeavors.[1] The more general methods for their preparation have been either fragmentation, or ring expansion, of smaller cyclic compounds. This is in stark contrast to five- and six-member ring strategies, where cycloadditions are seen as the most efficient way to rapidly construct systems with high yields and stereoselectivities.[2]

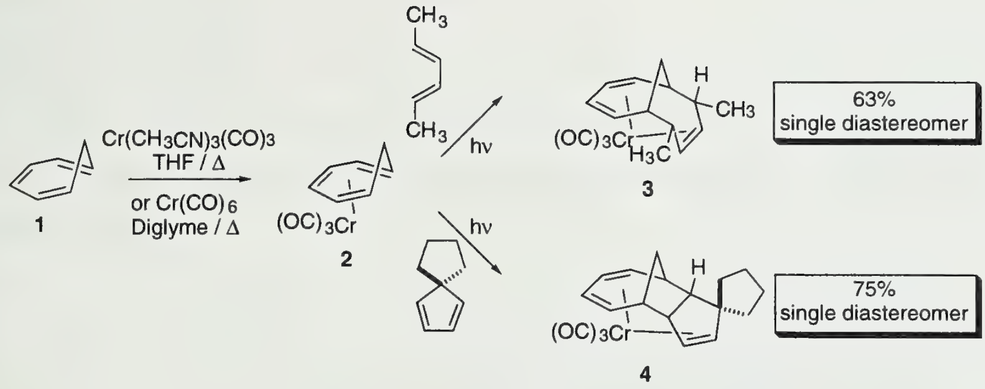
Hoffmann and Woodward[3] predicted in 1965 that higher-order, concerted cycloadditions were possible. This was soon followed by reports of a successful [6 + 4] cycloaddition between tropone and cyclopentadiene,[4] and a [6 + 2] cycloadduct between cycloheptatriene and benzyne.[5] A more thorough study by Houk and Woodward[6] showed that higher-order cycloadditions are inherently inefficient, due to competition with preferred reactions, such as [4 + 2] cycloadditions.

Attaching the triene and trienophile components creates an intramolecular pathway and generates the higher-order cycloadducts with much greater efficiency, due to there being fewer degrees of freedom along the reaction coordinate. This strategy was first utilized by Funk,[7] who in 1986 reported thermally induced [6 + 4] cycloadditions between tropone and dienes connected by a three-carbon tether. At about the same time, Feldman[8] discovered an acid-catalyzed intramolecular [6 + 2] cycloaddition, which he later used to synthesize the marine sesquiterpene (±)-Dactylol. All of these reactions exhibit high yields and periselectivities, with the major challenge being the synthesis of the tethered triene-trienophile precursor.

Complexation of both components to a transition metal would also render the cycloaddition intramolecular, but obviate the need for a tedious synthesis of the precursor. Attempts to combine iron-complexed cycloheptatriene with 2π components gave low yields of [6 + 2] adducts.[9] Similar results were seen with $TiCl_4$-Et_2AlCl[10] and molybdenum[11] promoted cycloadditions. These early attempts were plagued with poor yields and moderate selectivities. About 15 years ago, Kreiter discovered that chromium(0) effectively promotes the formal [6+4][12] and [6+2][13] cycloadditions of 1,3,5-cycloheptatriene with trienophiles (Scheme I). The cycloadditions normally produce single diastereomers in high yields, and the reaction is amenable to catalysis and asymmetric induction. Using this methodology, one can quickly construct bicyclic systems with a wide range of functionality and a high degree of stereocontrol.

Copyright © 1995 by Mark Seierstad

SCHEME I



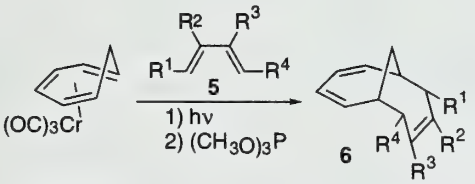
SCOPE

The stable triene-chromium complexes are formed by heating the triene with hexacarbonylchromium or trisacetonitriletricarbonylchromium.[14] Photochemical cycloadditions are performed in non-complexing solvents (e.g. hexane, toluene) at or near room temperature.[15] The reaction can also be effected thermally by heating the reagents (usually in a sealed tube) to between 140 and 160 degrees.[16] The tricarbonylchromium is easily removed from the [6 + 4] cycloadduct with trimethylphosphite or trimethylphosphine,[15] but the [6 + 2] products are usually recovered metal-free (because the [6 + 2] cycloadducts have only four π-electrons with which to bind a transition metal).[17]

Trienophile Variations

The chromium(0) promoted [6 + 4] cycloaddition is notable in that the electronic nature of the trienophile is largely irrelevant to the success of the reaction (see Table I). Both electron-rich and electron-deficient dienophiles give high yields of cycloadducts, and appear to react at equal rates.[16] The reaction is well suited to substitution at any position on the trienophile. In all instances, the products are exclusively the result of endo approach.

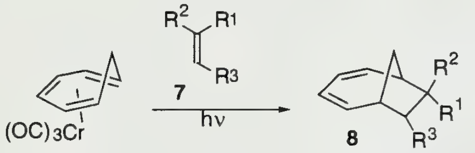
TABLE I



Entry	R^1	R^2	R^3	R^4	Yield (%)	Ref.
a	H	CH_3	CH_3	H	68	18
b	vinyl	H	H	H	65	19
c	CO_2CH_3	H	H	CH_3	96	16
d	CH_3	H	H	CH_3	86	16
e	OTMS	H	H	H	86	16
f	H	OTMS	H	H	82	20

Chromium(0) promoted [6 + 2] cycloadditions are much more sensitive to the electronic nature of the trienophile (Table II). Electron-donating groups render the reaction ineffectual (entry d); this might be due to the trienophile being bound too strongly to the metal center. However, a wide range of electron-deficient alkenes lead to cycloadducts, again with exclusive endo-selectivity. Also reactive are ketenes and alkynes (entries e,f). The reaction proceeds even with multiple substitution at bond-forming carbons (entry f) to form quaternary centers.

TABLE II

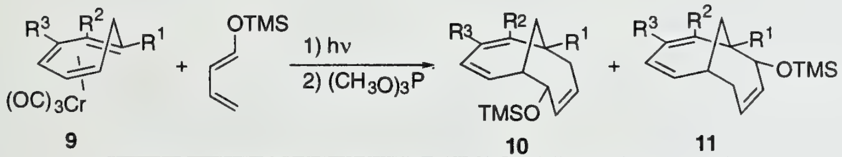


Entry	R^1	R^2	R^3	Yield (%)	Ref.
a	CO_2Et	H	H	92	21
b	H	CH_3	CO_2Et	58	21
c	SO_2Ph	H	H	49	17
d	OBu	H	H	0	21
e	Ph—≡—Ph			80	22
f	$Ph_2{=}\bullet{=}O$			50	23

Triene Variations

Rigby[16] has studied the regiochemical nature of [6 + 4] cycloadditions by using various substituted trienes (Table III). With [6 + 4] cycloadditions, acceptable selectivity, if seen at all, is displayed only with C1 substituted trienes (entry a) which suggests that it may be a result of steric interactions. Other modifications (entries b-e) produce no electronic or steric bias during the reaction.

TABLE III

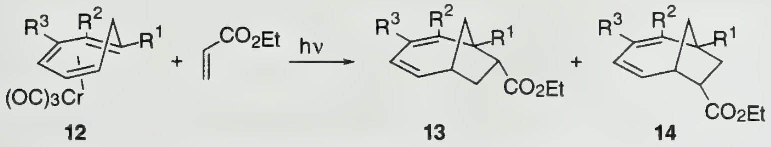


Entry	R^1	R^2	R^3	Yield (%)	Ratio of 10 : 11
a	OCH_3	H	H	38	10 only
b	CO_2CH_3	H	H	22	1 : 1
c	H	OCH_3	H	89	1 : 1
d	H	CO_2CH_3	H	75	1 : 1
e	H	H	OCH_3	79	1 : 1

The case of [6 + 2] cycloadditions is more complex (Table IV).[17] Substituents of diverse electronic effects afford high yields of products, but often with no selectivity. The exception is with C1 substituted trienes, which react with ethyl acrylate to give high yields of only the head-to-head adducts (**13a** and **13b**).

Examples in the literature are too few to draw general conclusions, except that C1 substituted trienes might have an electronic bias for head-to-head approach.

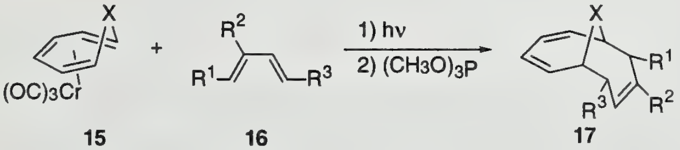
TABLE IV



Entry	R^1	R^2	R^3	Yield (%)	Ratio of 13 : 14
a	OCH_3	H	H	81	13 only
b	CH_3	H	H	87	13 only
c	H	CO_2CH_3	H	67	1 : 1
d	H	H	OCH_3	80	1 : 4
e	H	H	CO_2Et	80	13 only

Rigby[16] has also performed [6 + 4] cycloadditions with heteroatomic substituents at position seven (Table V). The results show that these trienes are amenable to higher-order cycloadditions. The N-(methoxycarbonyl)azepine complex, **15a,** readily reacts with both electron-rich and electron-deficient trienophiles to give the cycloadducts in high yields. The sulfone complex, **15c**, however, shows for the first time an electronic preference in the chromium(0) mediated [6 + 4] cycloaddition. Yields are much lower in those reactions with electron-deficient trienophiles than with electron-rich substrates. Unfortunately, the electronic nature of this triene has not been studied further with electron-deficient alkenes which seem to show regiochemical preferences due to electronic effects.

TABLE V



Entry	X	R^1	R^2	R^3	Yield (%)
a	$N\text{-}CO_2CH_3$	H	OTMS	H	87
b	$N\text{-}CO_2CH_3$	CH_3	H	CO_2CH_3	83
c	SO_2	OTMS	H	H	65
d	SO_2	CO_2CH_3	H	H	38
e	SO_2	CO_2CH_3	CO_2CH_3	H	0

MECHANISM

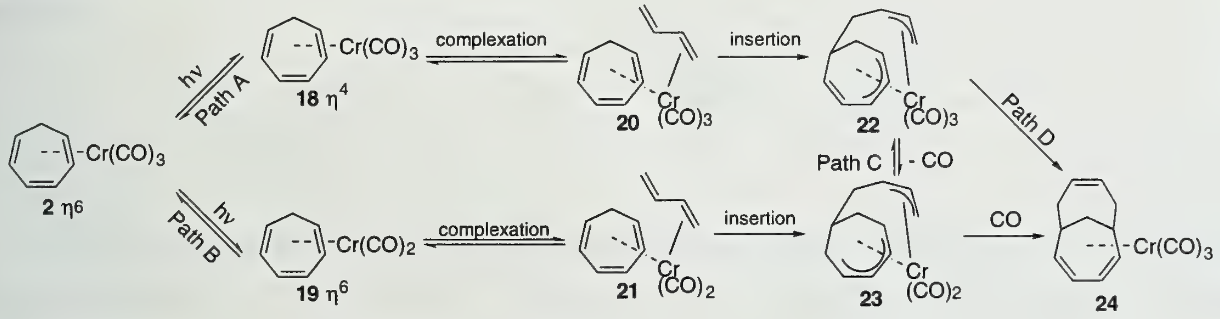
The mechanism of chromium(0) mediated [6 + 4] and [6 + 2] cycloadditions is still controversial, but some common steps are present in the various proposals (Scheme II). All the evidence suggests these are stepwise, formal cycloadditons. Except for the fact that electron-rich trienophiles do not participate in the [6 + 2] cycloaddition,[17] the two reactions are almost identical in efficiency, nature of allowable

substrates, and stereochemical outcome, suggesting that they may share many common mechanistic features.

It is known that the quantum yield (defined as the moles of product formed divided by the moles of photons absorbed) of the [6 + 2] cycloaddition is less than 1%.[24] This indicates that the photochemical step is readily reversible. η^6-Polyene-M(CO)$_3$ complexes, upon irradiation, can either undergo "hapticity slippage" from η^6 to η^4 (path A), or eject a molecule of CO (path B).[25] The low quantum yield suggests that a highly entropically favorable step, such as CO ejection, is not the key photochemical event.[24]

The hapticity slippage leaves chromium coordinatively unsaturated, which allows it to complex reversibly with the trienophile (complex **20**), setting the stage for bond insertion. The details of the path from **20** to **24** are still ambiguous, but the following observation lends insight. Kreiter[18] found that reaction of 2,3-dimethylbutadiene with the chromium complex of 8,8-dimethylheptafulvene gave a significant amount of **23** (presumably *via* path C). When subjected to a CO atmosphere, **23** proceeds with ring closure to give the [6 + 4] adduct **24**. No other proposed intermediates have been observed, and it is not known if **22** can go directly to **24** (path D).

SCHEME II

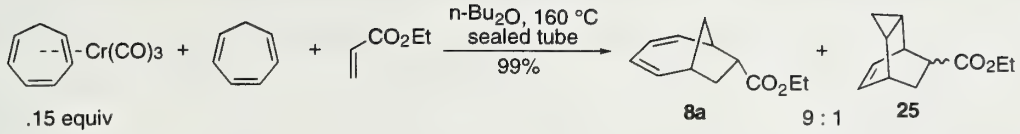


CATALYTIC CYCLOADDITIONS

It was mentioned previously that chromium(0)-mediated higher-order cycloadditions proceed with thermal activation as well as photochemical, and also that the transition metal is easily removed from the cycloadduct ligand. These features suggest that the reaction might proceed with a catalytic amount of chromium, which would reduce the amount of costly and toxic hexacarbonylchromium needed to prepare the substrates.

In [6 + 2] cycloadditions with alkenes as trienophiles, the diene cycloadducts are recovered metal-free; such cycloadditions are well-suited for catalysis. Rigby[17] found that thermal addition of ethyl acrylate to cycloheptatriene (Scheme III), with only 15 mol% of the complexed triene, gives a 9 to 1 mixture of easily separable products in quantitative yield. The minor product is the normal, metal-free cycloadduct (formed *via* electrocyclic ring-closure, followed by [4 + 2] cycloaddition).

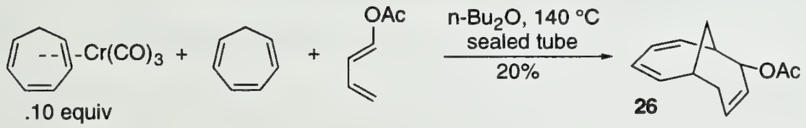
SCHEME III



For a catalytic [6 + 4] cycloaddition (or [6 + 2] with an alkyne) to be possible, the transition metal center must be lost from the triene-cycloadduct during the course of the reaction. Sheridan reported[22] that the irradiation of cycloheptatriene-tricarbonylchromium in the presence of diphenyl acetylene at room temperature produced, in addition to the [6 + 2] cycloadduct, a small amount of hexacarbonylchromium (observed by IR), presumably after decomplexation of the product. This was the first evidence of triene cycloadducts being decomplexed during the reaction.

Photochemical [6 + 4] cycloadditions do not normally give metal-free products (which is necessary to enter a catalytic cycle), but thermally induced reactions can.[16] A catalytic [6 + 4], performed in the same manner as the catalytic [6 + 2] above, gave low yields of a single cycloadduct (Scheme IV).[16] The low turnover may be an inherent problem, due to the stability of the chromium-cycloadduct complex.

SCHEME IV

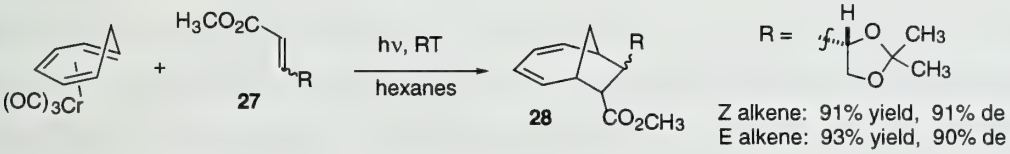


In both catalytic cycloadditions, the normally red reaction mixture turned green upon completion, suggesting the presence of chromium(III). While the [6 + 2] cycloaddition lends itself well to catalysis, nore work is needed with the [6 + 4] version, possibly using more labile chromium complexes.

ASYMMETRIC CYCLOADDITIONS

There have been a few efforts towards asymmetric [6 + 4] and [6 + 2] cycloadditions, all involving he use of chiral auxiliaries. High levels of asymmetric induction can be realized with chiral trienophiles uch as **27**, which give excellent yields of [6 + 2] adducts (Scheme V).[21]

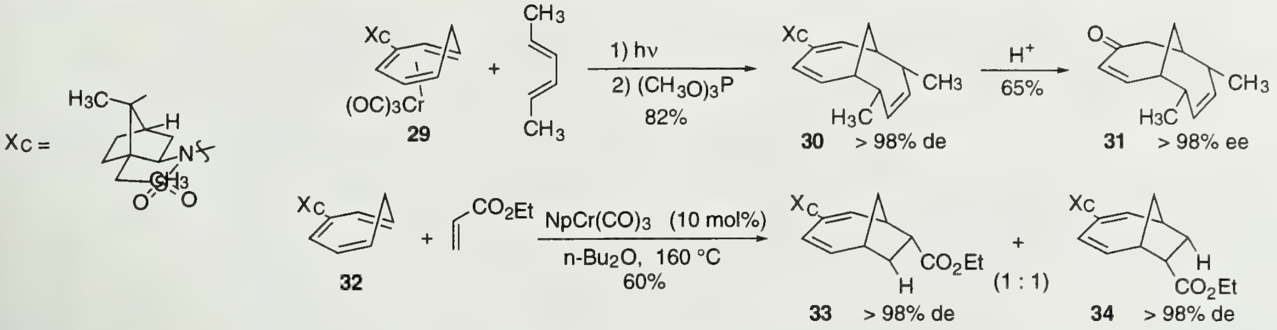
SCHEME V



Very recently, Rigby[26] announced preliminary work with chiral trienes. The 10-camphorsultam ubstituted cycloheptatriene (**29**) gives excellent asymmetric induction in both [6 + 4] and [6 + 2] ycloadditions (Scheme VI). Also, for the first time, a catalytic asymmetric [6 + 2] cycloaddition was

achieved, using tricarbonyl(naphthalene)chromium(0) as the source of metal center (the two regioisomeric cycloadducts **33** and **34** are easily separated by column chromatography).

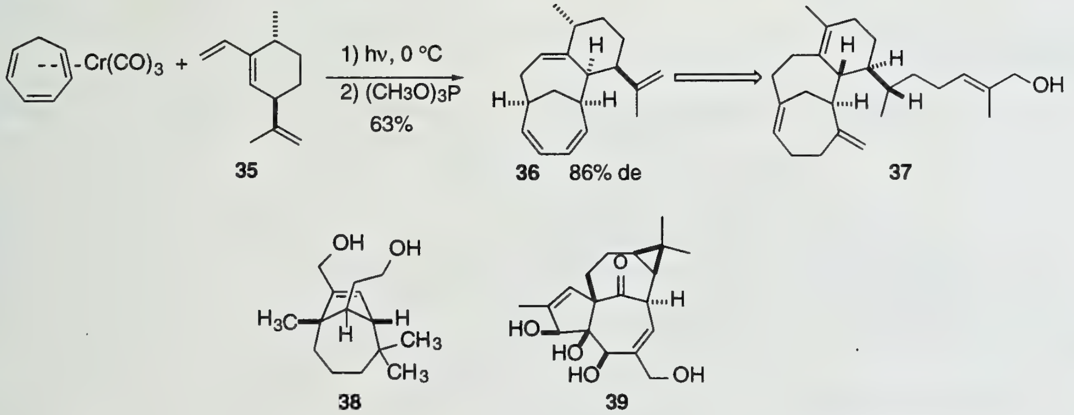
SCHEME VI



APPLICATIONS IN NATURAL PRODUCT SYNTHESIS

Although most of the work in chromium(0) mediated higher-order cycloadditions has been methodological, there has been work toward the total synthesis of natural products. Rigby[20] used optically pure trienophile **35** to selectively synthesize cycloadduct **36**, which could conceivably be elaborated to cerorubenol I (**37**). Studies directed toward the synthesis of the natural products secolongifolenediol (**38**)[21] and ingenol (**39**)[27] have been reported.

SCHEME VII



CONCLUSIONS

Higher-order cycloadditions are quick and efficient ways to rapidly construct eight- and ten-membered rings. Using chromium(0) to temporarily connect the two substrates allows formal [6 + 4] and [6 + 2] cycloadditions to be performed with excellent yield and stereoselectivity, and effectively shuts down [4 + 2] and other competing cycloadditions. A wide range of substrates gives the cycloadducts in high yields, including highly substituted trienophiles and heteroatomic substituted trienes. The reactions are often amenable to catalysis and high levels of asymmetric induction. Chromium(0)-mediated higher-order cycloadditions have a great deal of potential to become standard synthetic tools.

REFERENCES

1. Petasis, N. A.; Patane, M. A. *Tetrahedron* **1992**, *48*, 5757.
2. *Comprehensive Organic Synthesis*; Trost, B. M., Fleming, I., Eds.; Pergamon Press: Oxford, 1991; Vol. 5.
3. Hoffmann, R.; Woodward, R. B. *J. Am. Chem. Soc.* **1965**, *87*, 2046.
4. (a) Cookson, R. C.; Drake, B. V.; Hudec, J.; Morrison, A. *J. Chem. Soc. Chem. Commun.* **1966**, 15. (b) Itô, S.; Fujise, Y; Okuda, T.; Inoue, Y. *Bull. Chem. Soc. Jpn.* **1966**, *39*, 1351.
5. Tabushi, I.; Yamada, H.; Yoshida, Z.; Kuroda, H. *Tetrahedron Lett.* **1971**, 1093.
6. Houk, K. N.; Woodward, R. B. *J. Am. Chem. Soc.* **1970**, *92*, 4143.
7. Funk, R. L.; Bolton, G. L. *J. Am. Chem. Soc.* **1986**, *108*, 4655.
8. (a) Feldman, K. S.; Come, J.-H.; Freyer, A. J.; Kosmider, B. J.; Smith, C. M. *J. Am. Chem. Soc.* **1986**, *108*, 1327. (b) Feldman, K. S.; Come, J.-H.; Kosmider, B. J.; Smith, P. M; Rotella, D. P.; Wu, M.-J. *J. Org. Chem.* **1989**, *54*, 592. (c) Feldman, K. S.; Wu, M.-J.; Rotella, D. P. *J. Am. Chem. Soc.* **1990**, *112*, 8490.
9. (a) Ward, J. S.; Petit, R. *J. Am. Chem. Soc.* **1971**, *93*, 262. (b) Davis, R. E.; Dodds, T. A.; Hseu, T.-H.; Wagnon, J. C.; Devon, T.; Tancrede, J.; McKennis, J. S.; Petit, R. *J. Am. Chem. Soc.* **1974**, *96*, 7562.
10. Mach, K.; Antropiusová, H.; Sedmera, P.; Hanus, V.; Turecek, F. *J. Chem. Soc. Chem. Commun.* **1983**, 805.
11. Bourner, D. G.; Brammer, L.; Green, M.; Moran, G.; Orpen, A. G.; Reeve, C.; Schaverien, C. J. *J. Chem. Soc. Chem. Commun.* **1985**, 1409.
12. Özkar, S.; Kurz, H.; Neugebauer, D.; Kreiter, C. G. *J. Organomet. Chem.* **1978**, *160*, 115.
13. Kreiter, C. G.; Michels, E.; Kurz, H. *J. Organomet. Chem.* **1982**, *232*, 249.
14. (a) Able, E. W.; Bennett, M. A.; Burton, R.; Wilkinson, G. *J. Chem. Soc.* **1958**, 4559. (b) Pauson, P. L.; Todd, K. H. *J. Chem. Soc. (C)* **1970**, 2315.
15. Kreiter, C. G. *Adv. Organomet. Chem.* **1986**, *26*, 297.
16. Rigby, J. H.; Ateeq, H. S.; Charles, N. R.; Cuiciat, S. V.; Ferguson, M. D.; Henshilwood, J. A.; Krueger, A. C.; Ogbu, C. O.; Short, K. M.; Heeg, M. J. *J. Am. Chem. Soc.* **1993**, *115*, 1382 and references therein.
17. Rigby, J. H.; Ateeq, H. S.; Charles, N. R.; Henshilwood, J. A.; Short, K. M.; Sugathapala, P. M. *Tetrahedron* **1993**, *49*, 5495.
18. Michels, E.; Sheldrick, W. S.; Kreiter, C. G. *Chem. Ber.* **1985**, *118*, 964.
19. Kreiter, C. G.; Kurz, H. *Chem. Ber.* **1983**, *116*, 1494.
20. Rigby, J. H.; Ateeq, H. S. *J. Am. Chem. Soc.* **1990**, *112*, 6442.
21. Rigby, J. H.; Henshilwood, J. A. *J. Am. Chem. Soc.* **1991**, *113*, 5122.
22. Chaffee, K.; Sheridan, J. B.; Aistars, A. *Organometallics* **1992**, *11*, 18.
23. Rigby, J. H.; Ahmed, G.; Ferguson, M. D. *Tetrahedron Lett.* **1993**, *34*, 5397.
24. Fischler, I.; Grevels, F.-W.; Leitich, J.; Özkar, S. *Chem. Ber.* **1991**, *124*, 2857.
25. For CO ejection, see (a) Wrighton, M. *Chem. Rev.* **1974**, *74*, 401. For "hapticity slippage" see (b) O'Connor, J. M.; Casey, C. P. *Chem. Rev.* **1987**, *87*, 307.
26. Rigby, J. H.; Sugathapala, P.; Heeg, M. J. *J. Am. Chem. Soc.* **1995**, *117*, 8851.
27. Rigby, J. H.; Moore, T. L.; Rege, S. *J. Org. Chem.* **1986**, *51*, 2398.

COMPARISON OF METHODOLOGIES LEADING TO 1-ARYLNAPHTHALENE LIGNAN LACTONES

Reported by Michael E. Koscho

October 12, 1995

INTRODUCTION

Lignans are a class of natural products derived from vascular plants, and are closely related to lignin, a major component of cell walls along with polyoses.[1] Lignin, a polymeric substance, has as its main building blocks oxygenated derivatives of cinnamyl alcohol. Lignans also have these same building blocks as starting materials and they are dimeric structures formed from β to β' linkage of the two aryl propanoid moieties. Dimers which do not have the β to β' linkage are referred to a neo-lignans. Lignans can be subdivided into six major classes according to the oxidation state and further linkages of the side chain.[2,3]

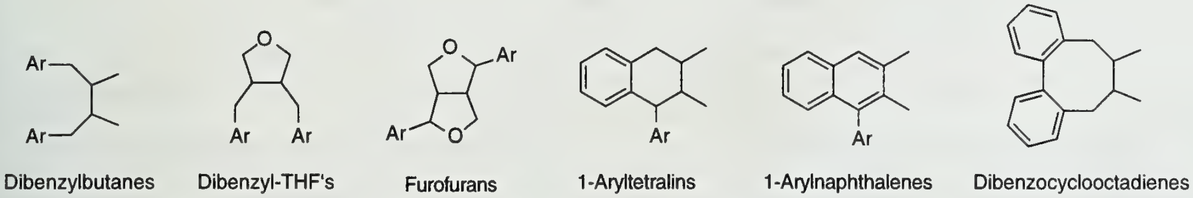


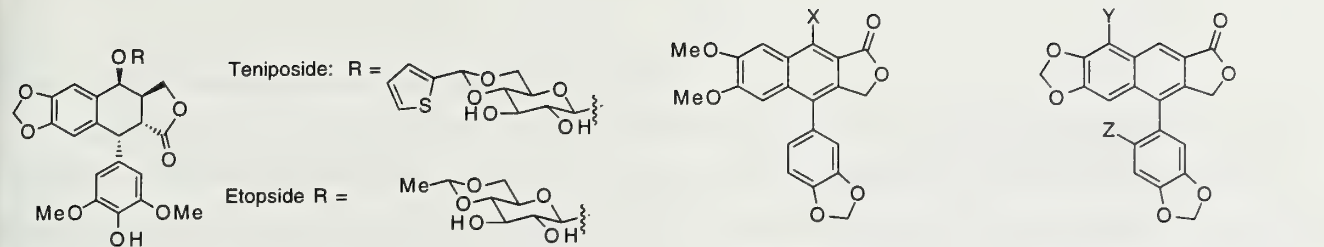
Figure 1. Six major subdivisions of lignans.

The focus of this review is lignans belonging to the 1-arylnaphthalenes. The first naturally occurring arylnaphthalene lignan isolated was diphyllin in 1961,[4] even though its structure was not properly elucidated until 1969.[5] Other arylnaphthalene lignan derivatives had been synthesized prior to this time, usually as by-products or as an aid in proving identities of aryltetralins. Over forty naturally occuring 1-arylnaphthalene lignans are now known, the majority of these (> 90%) are found as lactones.[2,3]

BIOLOGICAL PROPERTIES

Lignans display a wide range of biological activities. They have been found to be the active principle responsible for the activity of some folk-medicinal remedies.[6] Low incidence of cancer among vegetarians and people consuming a traditional Japanaese diet has been correlated to the high amounts of lignans in these foods.[7] A few lignans are presently used clinically. For example, the aryltetralin lignans etoposide and teniposide, which are derivatives of podophyllotoxin, are used as chemotherapy agents.[8] Closely related arylnaphthalenes, such as diphyllin and justicidin A, have also been shown to possess cytotoxic and cytostatic properties.[9] These two lignans along with justicidin B also show piscicidal activity.[6] Prostalidins A, B and C act as mild depressants.[10] Recently, synthetic derivatives of arylnaphthalene lignans have been shown to be promising in the development of novel anitasthma and antiinflammation agents.[11]

Copyright © 1995 by Michael E. Koscho



X = OH: Diphyllin
X = OMe: Justicidin A
X = H: Justicidin B

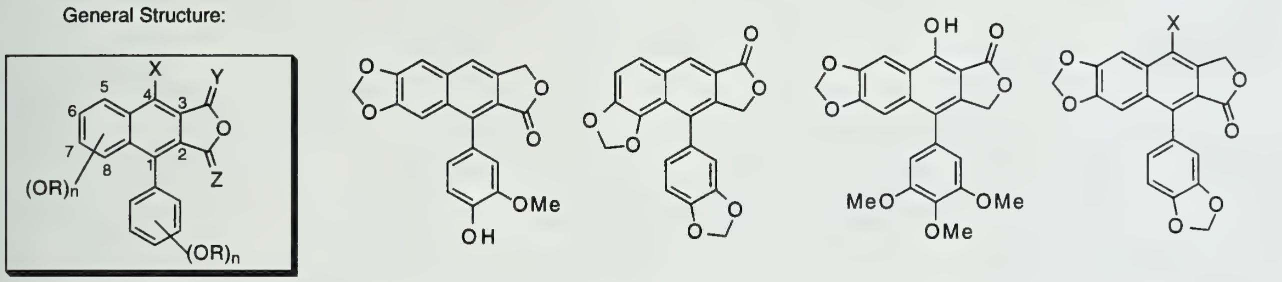
Y = OMe, Z = H: Prostalidin A
Y = OMe, Z = Me: Prostalidin B
Y = H, Z = H: Prostalidin C

Figure 2. Some biologically active lignans.

SYNTHESIS

Synthesis has played an important role in characterizing many of the arylnaphthalene lignans. With the low yields of material generally obtained from plant sources and with the possibility of discovering novel clinical agents, recent synthetic efforts have been directed toward developing efficient methodologies which can lead to a wide range of products with defined regiochemistry.

Any synthesis of these compounds must successfully take into account a few regiochemical concerns. First, positioning of the oxygenated substituents on the aryl rings must be controlled. Since all syntheses described herein begin with substituted phenyl rings, regioselective ring closure is a must. Secondly, syntheses which can be easily adapted to allow a 4-hydroxy substituent to be introduced on the naphthyl ring is desired since many of the natural products have this functionality. Finally, regioselective lactone formation is desired. Natural arylnaphthalene lignan lactones show both type I and type II lactones.


General Structure:

X = H: 4-deoxy; X = OH: 4-hydroxy
Y = O, Z = H_2: type I lactone
Y = H_2, Z = O: type II lactone

Chinensin

Helioxanthin

Dehydropodophyllotoxin

X = H: Taiwanin C
X = OH: Taiwnain E

Figure 3. General structure and some naturally occurring 1-arylnaphthalene lignans.

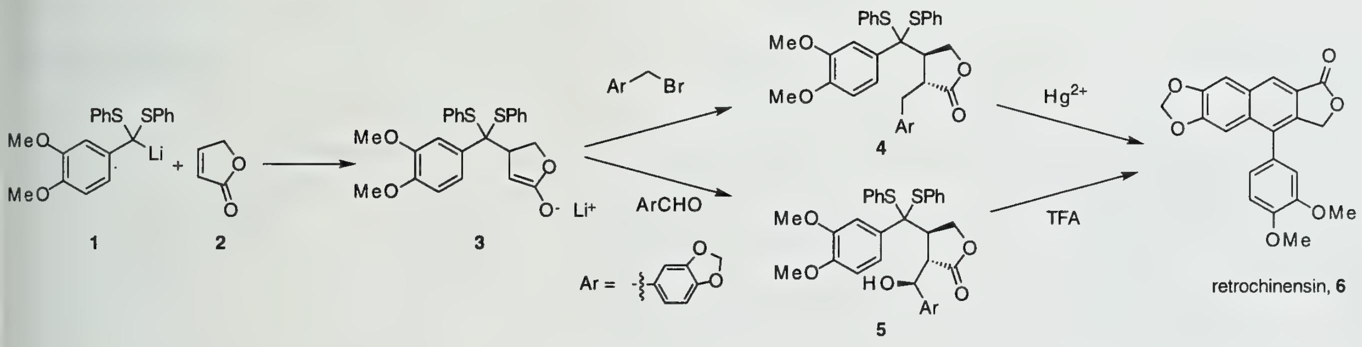
Many strategies have been developed for the elaboration of the 1-arylnaphthalene skeleton. These include: cyclization of the di-aryl Stobbe condensation products,[12] or aryl propiolic anhydrides,[13] addition of aryllithiums to naphthyl oxazolines,[14] Michael initiated ring closures utilizing dithianes[15,16] or silylated cyanohydrins,[17] and Diels-Alder reactions with quinodimethanes,[18] or 1-arylisobenzofurans.[19,20,21] The focus of this review will be on Michael additions followed by ring closures of dithianes or silylated cyanohydrins, cyclizations utilizing aryl propiolic anhydrides and Diels-Alder cyclizations utilizing 1-arylisobenzofurans. These methods offer distinct advantages in terms of controlling regiochemistry, simplicity of procedures or the ability to access a wide range of naturally occurring lignans through small procedural changes.

Michael Additions Followed by Ring Closures

This method uses a carbonyl synthon as the Michael donor which adds to a Michael acceptor with the resulting enolate being utilized as a nucleophile in an aldol condensation or an S_N2 displacement. This tandem sequence allows the arylnaphthalene skeleton to be constructed in a one-pot procedure. Depending on what is used as the carbonyl synthon and the protocol for removal, the intermediate adduct can be elaborated to either the 4-hydroxy or 4-deoxy lactones. The strategies developed so far do suffer from the fact that not one method is capable of leading to both types of lactones.

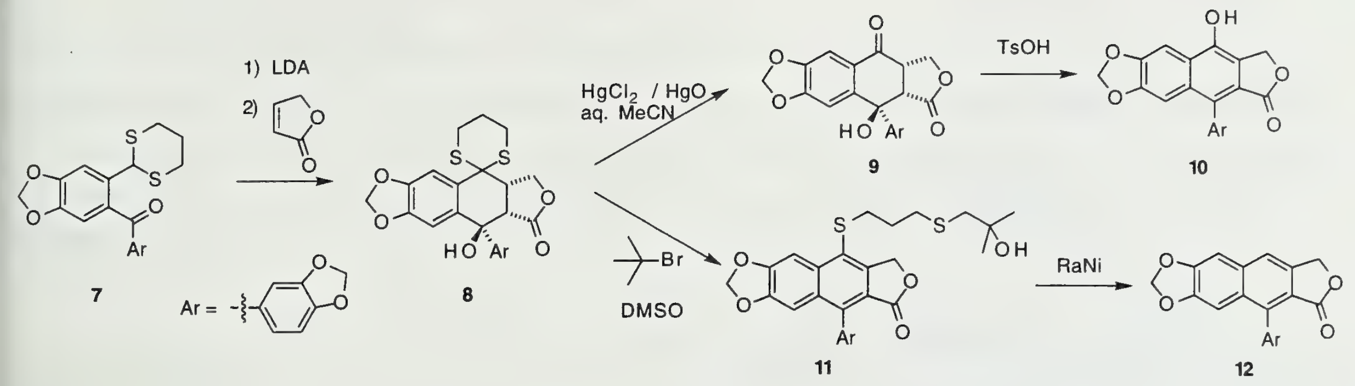
Originally applied to arylnaphthalenes by Pelter and Ward using diphenythio acetals as the Michael donor with ring closure mediated by mercury(II) cations, this method leads to only the 4-deoxy type I lactones.[15] Condensation of the Michael product (**3**), formed by addition of butenolide (**2**) to diphenylthio acetal (**1**), with an aromatic aldeyde leads to compound (**5**), formed as a single diastereomer. Reaction of intermediate (**3**) with a ring-oxygenated benzyl bromide derivative leads to compound (**4**), also formed as a single diastereomer. Intermediates (**4**) and (**5**) led to the same type I lactone (**6**) under their respective cyclization conditions. The inability to convert either intermediate to the type II lactone, or to the 4-hydroxy compound, limits this method. Typical yields of (**6**) are in the range of 35 - 45% from (**1**) using the mercury (II) cyclization route and around 60% for the tandem Michael-aldol-cyclization sequence.

SCHEME I



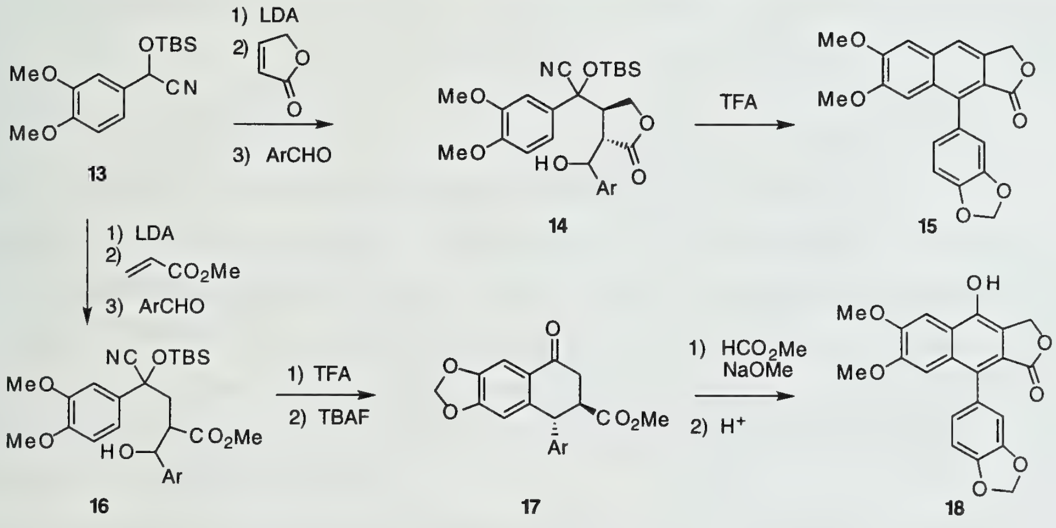
Harrowven and co-workers have developed a similar methodology which utilizes 1,3-dithianes as opposed to diphenylthio acetals.[16] Tandem Michael-aldol condensation of the dithiane (**7**) leads to the intermediate (**8**), which was formed as a single diastereomer. Deprotection of (**8**) with mercury(II), followed by acidic dehydration, leads to the 4-hydroxy type II lactone (**10**) in 21-49% yield from the dithiane (**7**). The intermediate (**8**) can also be converted to the 4-deoxy type II lactone. Treating (**8**) with the *tert*-butyl / dimethyl sulfoxide reagant [22] activates the dithiane for elimination, leading to (**11**) which can be desulfurized with Raney nickel to yield (**12**) in 37 - 55% yield from (**7**).

SCHEME II



A recent method developed by Iwasaki, Ohmizu and co-workers utilizes silylated cyanohydrins as the carbonyl anion synthon.[17] Tandem Michael addition of butenolide to the deprotonated silyl cyanohydrin (**13**) followed by condensation with an aryl aldehyde leads to the intermediate (**14**) as a mixture of four diastereomers. Intermediate (**14**) can be converted directly to the 4-deoxy type II lactone (**15**) by treatment with acid, with yields of 62 - 71% from (**13**). In order to access the 4-hydroxy type II lactone (**18**), methyl acrylate was used as the Michael acceptor. Acid catalyzed cyclization followed by deprotection of the silylated cyanohydrin lead to the ketone (**17**), which after Clasien condensation with methyl formate and acidic lactonization led to the 4-hydroxy type II lactone (**18**). The yields for this procedure are in the range of 28 - 37% from (**13**).

SCHEME III

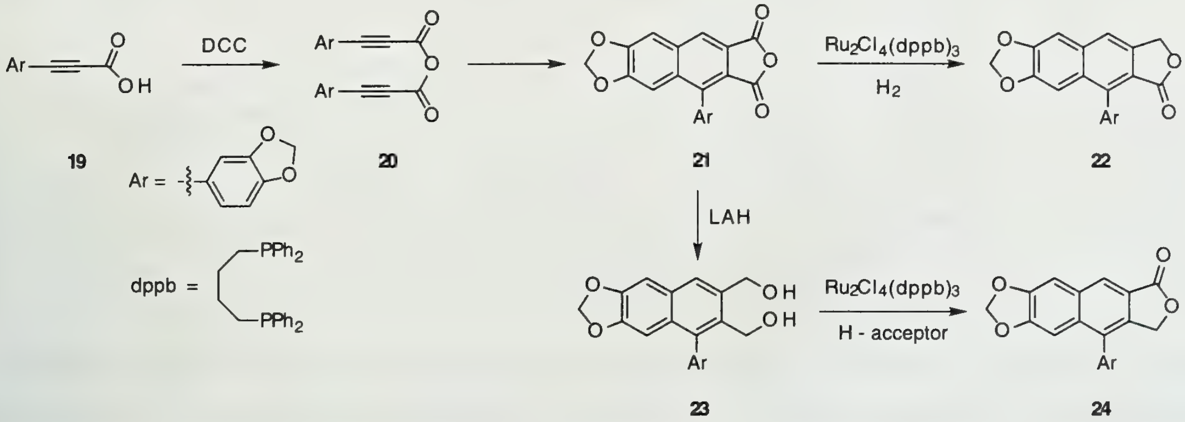


Coupling and Cyclization of Aryl Propiolic Acids

This method, pioneered by Stevenson and co-workers, is useful for synthesizing 4-deoxy arylnaphthalene lignan lactones where the substitution on both the phenyl and naphthyl rings are the same.[13] The aryl propiolic acid (**19**) can be coupled to give the anhydride (**20**) in good yields, generally around 50 - 60%. Intramolecular thermal cylization of the anhydride (**20**) leads to the arylnaphthalene

anhydride (**21**) which can be converted to either the type I or type II lactone regioselectively using the protocol developed by Saburi and co-workers.[23] Exposure of the anhydride (**21**) to the ruthenium catalyst leads to the type II lactone (**22**) with excellent regioselectivity (> 99 : 1) and yields of 82 - 89%. Alternatively, the anhydride (**21**) can be reduced to the diol (**23**) with lithium aluminum hydride (LAH). Subsequent reaction of the diol (**23**) with the ruthenium catalyst in the presence of a hydrogen acceptor leads to the type I lactone (**24**), also with excellent regioselectivity (> 98 : 2) and yields of 88 - 94%.

SCHEME IV



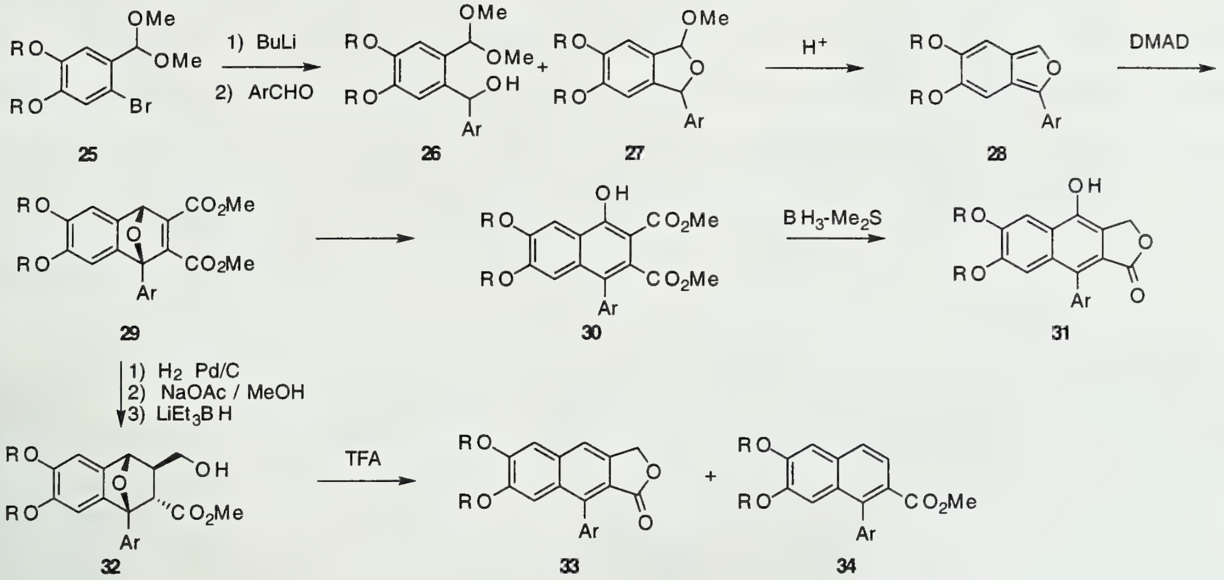
Diels-Alder Reactions of 1-Arylisobenzofurans

Each of these methods utilizes a substituted isobenzofuran as the diene. Diels-Alder reaction of an isobenzofuran leads to an ether linked bicyclic adduct which can be aromatized to the naphthalene under acidic conditions. The main difference among the methods is the substitution of the isobenzofuran and the protocol employed for its synthesis. Depending on the substitution of the isobenzofurans, the cycloadducts may be elaborated to either the 4-hydroxy or 4-deoxy naphthalenes. Using methods that have been developed for regioselective lactone formation makes this approach very powerful.

This method was originally applied to arylnaphthalenes by Rodrigo and co-workers.[19] Starting with the 2-brominated verataldehyde dimethyl acetal (**25**), lithium-halogen exchange followed by condensation with an aldehyde led to an inseparable mixture of (**26**) and (**27**). Treatment of this mixture with acid leads to the 1-arylisobenzofuran (**28**). Diels-Alder reaction with dimethyl acetylene dicarboxylate (DMAD) affords the diester which can selectively be reduced, leading to the 4-hydroxy type II lactone (**31**), with yields around 35% from (**25**).

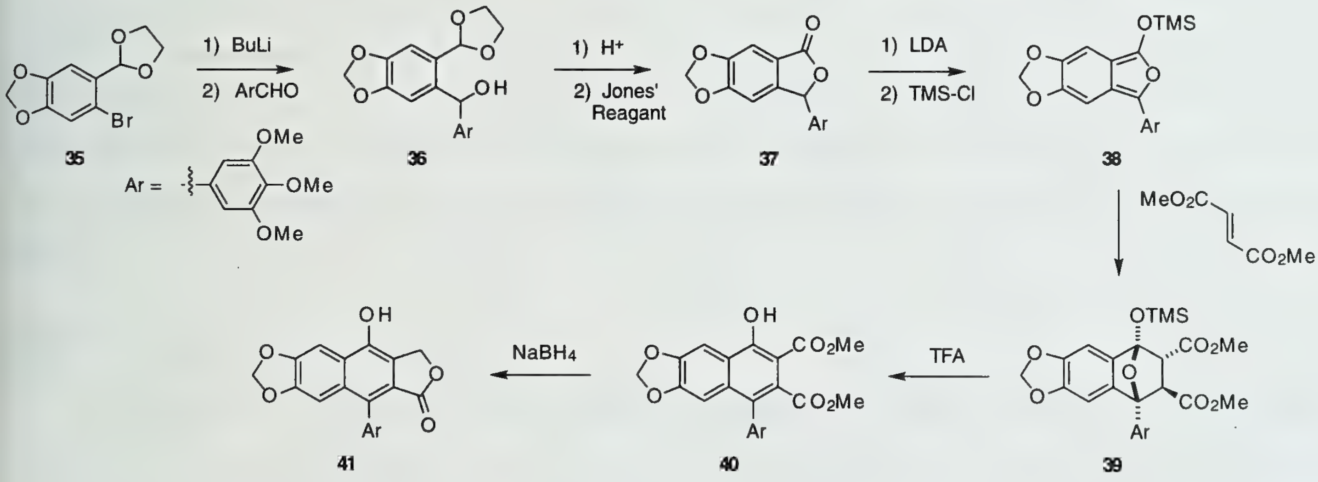
With a modification, the same cycloadduct can be used to synthesize the 4-deoxy counterparts. Trapping of the cycloadduct (**29**), hydrogenation, epimerization at C-3 and selective reduction of the ester leads to (**32**). Aromatization and lactonization in trifluoroacetic acid leads to a 1 : 1 mixture of (**33**) and (**34**). Typical yields are in the range of 20% from (**25**). Significant by-product formation, along with a lengthy sequence of reactions, limits the utility of this method.

SCHEME V



Another method involves generating the isobenzofuran from a phthalide to yield an α-silyloxy-arylisobenzofuran.[20] Treatment of the phthalide (**37**) with LDA, then trapping the isobenzofuran with trimethylsilyl chloride leads to (**38**). Diels-Alder reaction of (**38**) with dimethyl fumarate leads to the cycloadduct (**39**) which can be aromatized to yield the diester (**40**). The diester (**40**) can be selectively lactonized to yield the type II lactone (**41**), with typical yields in the range of 35 -40% from (**37**). This method is also limited in the number of products attainable.

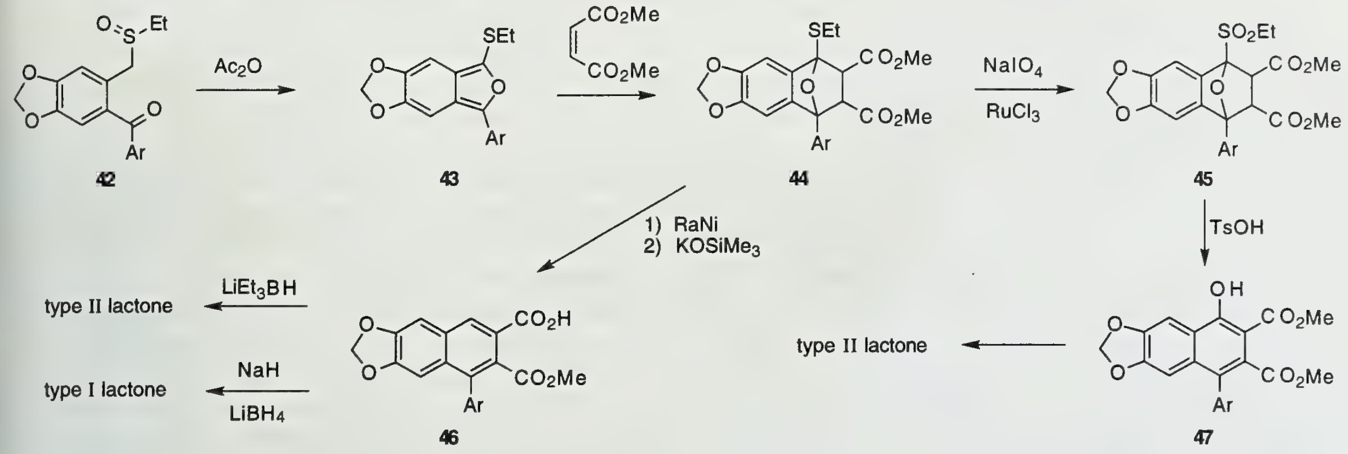
SCHEME VI



Recently, Padwa and co-workers have developed a tandem Pummerer-Diels-Alder synthesis of 1-arylnaphthalene lignans.[21] This method utilizes an intermediate α-thio-arylisobenzofuran (**43**), which is formed by intramolecular interception of the Pummerer intermediate produced by exposure of (**42**) to acetic anhydride. Following Diels-Alder reaction with dimethyl maleate, the cycloadduct can be converted to either the 4-hydroxy type II lactone or the 4-deoxy type I and II lactones. Conversion of the sulfide of

cycloadduct (**44**) to the sulfone activates it for elimination. Exposure of the sulfone (**45**) to acid leads to the oxygen retained 4-hydroxy diester (**47**) which, as seen previously, can be converted to the type II lactone. Yields of 65 - 70% are typical for the diester (**47**) from (**42**). Alternatively, desulfurization of the cycloadduct (**44**) followed by aromatization leads to the 4-deoxy diester which can be selectively hydrolized at the less hindered carbonyl with the bulky potassium trimethysilanolate.[24] The half-ester (**46**) can then be selectively reduced and lactonized to either the type I or type II lactone depending on choice of reducing reagant. Both procedures give yields in the 40 - 60% range from (**42**).

SCHEME VII



CONCLUSIONS

Many methods have been developed for regioselective synthesis of 1-arylnaphthalene lignan lactones. A few of these methods are capable of accessing a wide range of these natural products. Most of the methods presented go through highly functionalized intermediates which have found or will likely find application in stereoselective total syntheis of other lignans, especially the 1-aryltetralins.[2,3,25,26] Methodologies so far developed have also found application in synthesizing heterocyclic analogues of arylnaphthalene lignans.[27] Further developments in this field will undoubtedly include biological screening, and the development of structure-activity relationships of the library of 1-arylnaphthalenes now attainable.

REFERENCES

1. D. Fengel and G. Wegener, "Wood: Chemistry, Ultrastructure and Reactions," de Geyten, Berlin **1984**.
2. (a) "The Chemistry of Lignans," ed. C. B. S. Rao, Andhara University Press, Watair **1978**. (b) "Lignans, Chemical, Biological and Clinical Properties," eds. D. C. Ayres and J. D. Loike, Cambridge University, New York **1990**.
3. (a) R. S. Ward, *Chem. Soc. Rev.*, **1982**, *11*, 75. (b) D. A. Whiting, *Nat. Prod. Rep.*, **1985**, 2, 191. (c) D. A. Whiting, *Nat. Prod. Rep.*, **1987**, *4*, 499. (d) D. A. Whiting, *Nat. Prod. Rep.*, **1990**, *7*, 349. (e) R. S. Ward, *Nat. Prod. Rep.*, **1993**, *10*, 1. (f) R. S. Ward, *Nat. Prod. Rep.*, **1995**, *12*, 183.
4. T. Murakami and A. Matsushima, *J Pharm. Soc., Japan*, **1961**, *81*, 1596.
5. Z.-I. Horii, K. Ohkawa, S.-W. Kim and T. Momose, *Chem. Pharm. Bull.*, **1969**, *17*, 1878.

6. W. D. McRae and G. H. N. Towers, *Phytochemistry*, **1984**, *23*, 1207.
7. (a) H. Adlercreutz, H. Honjo, A. Higashi, T. Fostis, E. Hamalainen, T. Hasegawa and H Okada, *Am. J. Clin. Nutr.*, **1991**, *54*, 1093. (b) L. U. Thompson, P. Robb, M. Serraino and F. Cheung, *Nutr. Canc.*, **1991**, *16*, 43.
8. J. C. Yalowich, D. W. Fry and I. D. Goldman, *Cancer Res.*, **1982**, *42*, 3648.
9. (a) A. G. Gonzalez, V. Darias, G. Alonso, *Planta Med.*, **1979**, *36*, 200. (b) *Nat. Prod. Rep.*, **1986**, *49*, 348.
10. S. Ghosal, S. Banerjee and A. W. Frahm, *Chem. Ind.*, **1979**, 854.
11. Y. Ducharme, C. Brideau, D. Dube, C. C. Chan, J. P. Falgueyret, J. W. Gillard, J. Guay, J. H. Hutchinson, C. S. McFarlane, D. Riendeau, J. Scheigetz and Y. Girard, *J. Med. Chem.*, **1994**, *37*, 512.
12. D. C. Ayres, B. G. Carpenter and R. C. Denney, *J. Chem Soc. C*, **1965**, 3578.
13. (a) D. Brown and R. Stevenson, *Tet. Letters*, **1964**, 43, 3213. (b) D. Brown and R. Stevenson, *J. Org. Chem.*, **1965**, 30, 1759. (c) T. L. Holmes and R. Stevenson, *Tet. Letters*, **1970**, 49, 199. (d) T. L. Holmes and R. Stevenson, *J. Chem. Soc. C*, **1971**, 2091. (e) T. L. Holmes and R. Stevenson, *J. Org. Chem.*, **1971**, 36, 3450. (f) E. Block and R. Stevenson, *J. Org. Chem.*, **1971**, 36, 3453. (g) A. G. Gonzalez, J. P. Perez and J. M. Trujillo, *Tetrahedron*, **1978**, 34, 1011. (h) R. Stevenson and S. V. Weber, *J. Nat. Prod.*, **1989**, 52, 367. (i) P. T. Anastus and R. Stevenson, *J. Nat. Prod.*, **1991**, 54, 1687.
14. (a) A. I. Meyers and W. B. Avila, *J. Org. Chem.*, **1981**, *46*, 3881. (b) R. C. Andrews, S. J. Teague and A. I. Meyers, *J. Am. Chem. Soc.*, **1988**, *110*, 7854.
15. (a) A. Pelter, R. S. Ward, P. Satyanarayana and P. Collins, *Tet. Letters*, **1982**, *23*, 571. (b) A. Pelter, R. S. Ward, P. Satyanarayana and P. Collins, *J. Chem. Soc. Perkins I*, **1983**, 643. (c) A. Pelter, R. S. Ward, M. C. Pritchard and I. T. Kay, *J. Chem. Soc. Perkin 1*, **1988**, 1603. (d) J. F. Payack and D. R. Bender, *Org. Prep. Proc. Int.*, **1993**, *25*, 368.
16. (a) D. C. Harrowven, *Tet. Letters*, **1991**, *32*, 3735. (b) D. C. Harrowven and S. J. Dennison, *Tet. Letters*, **1993**, *34*, 3323. (c) D. C. Harrowven, *Tetrahedron*, **1993**, *49*, 9039.
17. (a) T. Ogiku, M. Seki, M. Takahashi, H. Ohmizu and T. Iwasaki, *Tet. Letters*, **1990**, *31*, 5487. (b) T. Ogiku, S. Yoshida, T. Kuroda, H. Ohmizu and T. Iwasaki, *SYNLETT*, **1992**, 651. (c) T. Ogiku, S. Yoshida, H. Ohmizu and T. Iwasaki, *J. Org. Chem.*, **1995**, *60*, 4585.
18. (a) J. Mann, S. E. Piper and L. K. P. Yeung, *J. Chem. Soc. Perkins I*, **1984**, 2081. (b) J. L. Charlton and T. Durst, *Tet. Letters*, **1984**, *25*, 5287.
19. (a) R. Rodrigo, *J. Org. Chem.*, **1980**, *45*, 4538. (b) H. P. Plaumann, J. G. Smith and R. Rodrigo, *J. Chem. Soc. Chem. Comm,*, **1980**, 354. (c) S. Osmund, C. St. Denis and R. Rodrigo, *J. Chem . Soc. Chem. Comm.*, **1980**, 995. (d) S. Horne and R. Rodrigo, *J. Chem. Soc. Chem. Comm.*, **1992**, 164.
20. (a) M. Iwao, H. Inoue and T. Kurashi, *Chem. Letters*, **1984**, 1263. (b) S. M. Gokhale, R. R. Joshi and N. S. Narasimhan, *Indian J. Chem.*, **1987**, *26B*, 1030.
21. (a) J. E. Cochran and A. Padwa, *Tet. Letters*, **1995**, *36*, 3495. (b) J. E. Cochran and A. Padwa, *J. Org. Chem.*, **1995**, *60*, 3938.
22. (a) C. Tenca, A. Dossena, R. Marchelli and G. Casnati, *Synthesis*, **1981**, 141. (b) G. A. Olah, A. K. Mehrotra and S. C. Narang, *Synthesis*, **1982**, 151.
23. Y. Ishii, T. Ikariya, M. Saburi and S. Yoshikawa, *Tet. Letters*, **1986**, *27*, 365.
24. E. D. Laganis and B. L. Chenard, *Tet. Letters*, **1984**, *25*, 5831.
25. (a) R. S. Ward, *Tetrahedron*, **1990**, *46*, 5029. (b) R. S. Ward, *Synthesis*, **1992**, 719.
26. (a) Kashima, M. Tanoguchi, M. Arimoto and H. Yamaguchi, *Chem. Pharm. Bull.*, **1991**, *39*, 192. (b) T. Ogiku, S.-I. Yoshida, T. Kuroda, M. Takahashi, H. Ohmizu and T. Iwasaki, *Bull. Chem. Soc. Jpn.*, **1992**, *65*, 3495. (c) A. Pelter, R. S. Ward, D. M. Jones and P. Maddocks, *J. Chem. Soc. Perkins I*, **1993**, 2621. (d) A. Pelter, R. S. Ward, L. Qianrong and J. Pis, *Tetrahedron: Asymmetry*, **1994**, *5*, 909. (e) A. Pelter, R. S. Ward and N. P. Storer, *Tetrahedron*, **1994**, *50*, 10829. (f) R. S. Ward, A. Pelter, M. I. Edwards and J. Gilmore, *Tetrahedron: Asymmetry*, **1995**, *6*, 843.
27. (a) T. Kuroda, M. Takahashi, T. Ogiku, H. Ohmizu, K. Kondo and T. Iwasaki, *J. Chem. Soc. Chem. Comm.*, **1991**, 1635. (b) S. W. McCombie, J. R. Tagat, W. A. Metz, D. Nazareno and M. S. Puar, *Tetrahedron*, **1993**, *49*, 8073. (c) T. Kuroda, M. Takahashi, T. Ogiku, H. Ohmizu, T. Nishitani, K. Kondo and T. Iwasaki, *J. Org. Chem.*, **1994**, 7353.

ÅCATALYTIC ASYMMETRIC HYDROGENATIONS USING NOVEL *C_2*-SYMMETRIC BIS(PHOSPHOLANE) LIGANDS

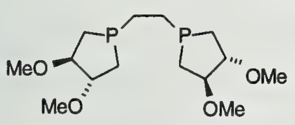
Reported by Marc Skaddan November 9, 1995

INTRODUCTION

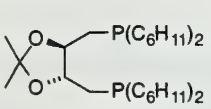
Since the discovery of homogeneous asymmetric catalysts in 1968 by Horner and co-workers,[1] there has been an explosion in the number of asymmetric phosphine ligands used for the hydrogenation of prochiral substrates. Most of these hydrogenation catalysts have utilized a C_2-symmetric ligand with at least two aryl groups attached to the phosphorus atom.[2] Since the aryl groups render the phosphorus relatively electron-deficient, these catalysts are generally classified as "electron-poor". On the other hand, catalysts with various alkyl groups bonded to the phosphorus are accordingly dubbed "electron-rich".[3] While much study has been done on successful electron-poor catalysts, these electron-rich catalysts has been left relatively unexplored,[3] and will be emphasized in this review.

BACKGROUND

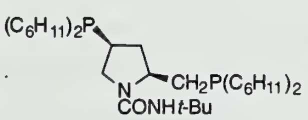
Recent hydrogenations using electron-rich phosphines have provided poor to good results. Brunner and co-workers in 1987 reported that the monodentate alkylphospholane ligand **1** reduced the olefin (*Z*)-*α*-(*N*-acetamido)cinnamic acid to (*R*)-*N*-acetylphenylalanine with 16.8% ee.[4] In 1989, Tani and co-workers were able to hydrogenate *α*-ketoamides and *α*-ketopantolactones with rhodium(0) complexes of Cy-diop (**2**) and (*R*)-Cycapp (**3**) with 66-77% ee.[5] In 1989, Myashita and co-workers reported that the rhodium complex of BICHEP (**4**) converted *α*-(benzamido)cinnamate to *N*-benzoyl-(*S*)-phenylalanine ethyl ester with 98% ee.[6] Since then **4** has been used to hydrogenate itoconic acid and its methyl ester with 93% and 95% ee, respectively.[7]



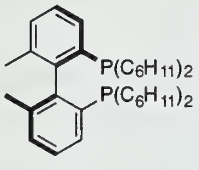
1



2



3

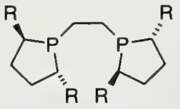


4

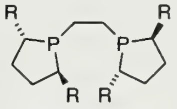
A breakthrough in electron-rich diphosphine hydrogenations came in 1990 when Burk and co-workers reported that good ee's were obtained by hydrogenation of methyl acetamidocinnamate and dimethyl itoconate with rhodium(I) complexes of 1,2-bis(phospholano)ethanes **5** and **6** (BPE ligands) under mild conditions.[8] Since then a similar set of ligand systems, 1,2-bis(phospholano)benzenes **7** and **8** (DuPHOS ligands) have been synthesized. Both ligand systems have been shown to give excellent enantioselectivities for a variety of substrates under mild conditions. The focus of this review will be on

Copyright © 1995 by Marc Skadden

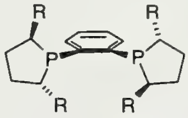
the unique properties of the BPE and DuPHOS ligand systems and their applications to the synthesis of a variety of enantiomerically enriched compounds.



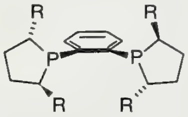
(*R*,*R*)-**5a**, R = Me
(*R*,*R*)-**5b**, R = Et
(*S*,*S*)-**5c**, R = *i*-Pr
(*R*,*R*)-**5d**, R = Pr



(*S*,*S*)-**6a**, R = Me
(*S*,*S*)-**6b**, R = Et
(*R*,*R*)-**6c**, R = *i*-Pr
(*S*,*S*)-**6d**, R = Pr



(*R*,*R*)-**7a**, R = Me
(*R*,*R*)-**7b**, R = Et
(*S*,*S*)-**7c**, R = *i*-Pr
(*R*,*R*)-**7d**, R = Pr



(*S*,*S*)-**8a**, R = Me
(*S*,*S*)-**8b**, R = Et
(*R*,*R*)-**8c**, R = *i*-Pr
(*S*,*S*)-**8d**, R = Pr

Characteristics and Preparation of Bis(phospholano) Ligands

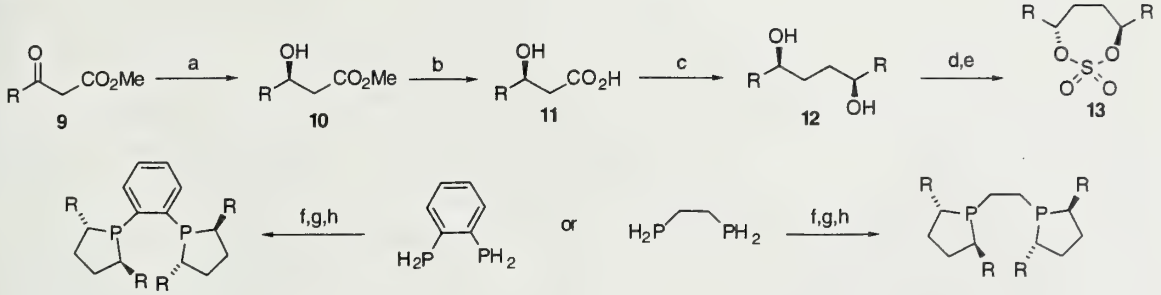
It would be ideal to have ligand systems with different backbone flexibilities, that is, different phosphorus to phosphorus linker pliability. The DuPHOS ligand systems have a 1,2-phenylene backbone which imposes more rigidity on the ligand than the ethane bridge on the BPE ligands.[8] This could reduce conformational mobility and increase the efficiency of chirality transfer.[9] However, in some substrates, such as ketones and β-keto esters, it has been shown that ligand flexibility appears to be important for catalysis to proceed.[5] Therefore, it would be beneficial to have two ligand systems, one with a conformationally mobile backbone, and one with a firm backbone, to "fine tune" a specific reaction to a substrate's demands.

In the majority of traditional electron-poor diphosphine complexes, the chirality induced by the ligand resides in its backbone, which is about 4-5 Å from the metal coordination site.[1b] The conformation of the chiral backbone promotes a chiral "face-edge" array of the aryl groups on the phosphorus. It is this array that transmits the chirality to the prochiral substrate.[1b] The DuPHOS and BPE ligand systems, with their chiral phospholane rings, provide an ordered C_2-symmetric environment that would not rely on secondary transfer of chirality from the aryl substituents, but instead directly interacts with the metal coordination sphere.[9]

Finally, varying the R groups on **5-8** would allow one to match the steric demands of various substrates without significantly varying the electronic nature of the metal center.[9] Varying the steric bulk on chiral diarylphosphines would inevitably change the ligand electronics.[9]

The preparation of **5-8** (Scheme 1) involves asymmetric hydrogenation of β-keto ester **9** to alcohol **10**, followed by Kolbe coupling of β-hydroxy carboxylic acid **11** to homochiral diol **12**. This is converted to the cyclic sulfate **13**, followed by the nucleophilic addition of the dilithium bis(phosphido)ethane or bis(phosphido)benzene to **13** to form the BPE and DuPHOS ligands, respectively.[3,9] Formation of 10 involves an electron-poor Ru-BINAP catalyst designed for β-keto ester reduction.[11] Interestingly, recent studies have shown that a ruthenium complex of **6c** is capable of hydrogenating ethyl isobutyrylacetate to ethyl (*R*)-3-hydroxy-4-methylpentanoate in 99% ee,[12] providing a rare example of catalyst self-generation (asymmetric ligand breeder cycle).[13]

Scheme 1[a]



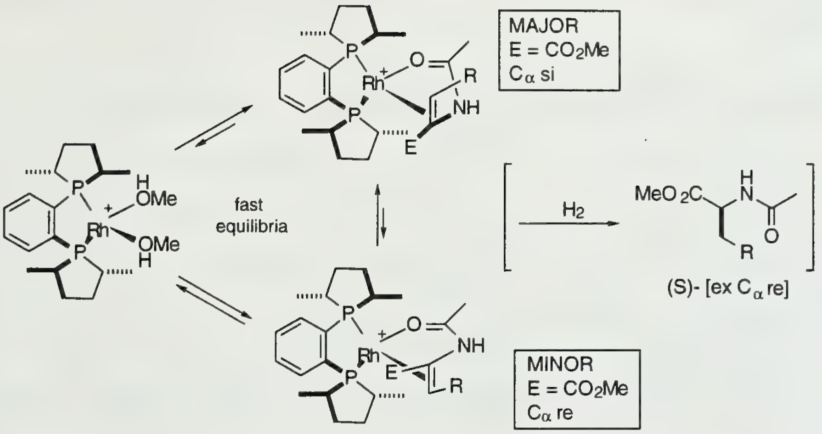
[a] Key (a) [Ru-((R)-BINAP)] cat, H_2 (100 atm); (b) KOH; (c) Kolbe coupling; (d) $SOCl_2$; (e) $RuCl_3$, $NaIO_4$; (f) 2 eq. *n*-BuLi; (g) **13** (2 eq); (h) 2 eq. *n*-BuLi

MECHANISM

Mechanistic work relating to the hydrogenation of prochiral substrates by chiral diphosphine ligands has been limited primarily to studies based on the most successful substrates, α-enamides, and the most successful catalysts, electron-poor chiral diphosphines (e.g. DIPAMP, CHIRAPHOS).[1] Although no detailed mechanistic studies have been done on complexes involving BPE and DuPHOS ligands, preliminary results suggest that these ligands exhibit the same general mechanism as that of their electron-poor counterparts.[14] Studies on electron-poor rhodium diphosphine catalysts show that complexation of a prochiral α-enamide to a chiral diphosphine (e.g. DIPAMP) yields two diastereomeric species, one present to a much greater extent (>95%) than the other.[14] Of the two, the minor complex is much more reactive than the major complex towards hydrogen gas, the rate-determining step at room temperature.[1a,14] Thus, the minor diastereomeric complex leads to the major product.

With respect to the BPE and DuPHOS ligands, **8a** was chosen for study. When an α-enamide was added to **8a**, ^{31}P NMR showed that two diastereomeric complexes were formed, one major and one minor (Scheme 2).[14] The major complex is bound to the *Si*-face of the α-enamide, while the minor complex is bound to the *Re*-face.[14] It is known that **8a** gives the *S*-product, a product that could be formed only from the *Re*-face complex. Thus, formation of the major enantiomer proceeds through the minor *Re*-face complex, rather than the major *Si*-face complex. The binding constant K of the α-enamide to **8a** was only in the 10^2 M^{-1} range, as opposed to previous values of 10^3-10^5 M^{-1} seen for related electron-poor diphosphine complexes.[1,14] This might account for the enhanced rates of reactions for hydrogenations of α-enamides with BPE and DUPHOS complexes, which generally proceed in an hour. This is in contrast to traditional electron-poor diphosphines, some of which can take up to 48 hours for complete conversion.[15] A lower binding constant K effectively facilitates conversion of the major complex to the more reactive minor complex.

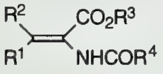
Scheme 2



SYNTHETIC UTILITY

α-Enamide Hydrogenations

Because many of the first successful uses of homogeneous asymmetric catalysts were done on α-acylaminoacrylic acids **14** and their derivatives, they have proven to be useful substrates to test the asymmetric induction of new catalyst systems.[1] Rhodium complexes of **7d** and **8d** have shown great promise in hydrogenating many α-enamides to their corresponding amino acids.[9]



14

As shown in Table 1, Rh-**7d** and Rh-**8d** give similar or greater ee's than those of their electron-poor counterparts when hydrogenating the α-enamide precursors of alanine, phenylalanine, and leucine. It is notable that the enantioselectivities do not change for the DuPHOS catalyst when the R^3 group is changed from a methyl to a hydrogen, or when the R^4 group is changed from a methyl to a phenyl.[9] Likewise, substituting the phenyl ring of dehydrophenylalanine does not change the enantioselectivity appreciably.[9] This is unlike many of the traditional diphosphine catalysts, whose enantioselectivities can change dramatically when the R groups in **14** are changed even slightly.[21]

It is significant that the *E* isomers of α-enamides (*E* = R^2-substituted, opposite amide group) are hydrogenated in essentially the same ee's as their *Z* counterparts with the Rh-**7d** and Rh-**8d**, since (*E*)-α-enamides are often reduced with much lower selectivity than (*Z*)-α-enamides.[7] Deuteration studies suggest that the high ee's are not caused by isomerization of the *E*-isomer to the *Z*-isomer during catalysis.[9] Hence, the mechanistic origin of the success with both isomers remains unclear.

Table 1. Hydrogenations of β-Substituted α-Enamides With 7d and 8d

Entry	Substrate (14)	Catalyst (Previous Best)	EE (%)	EE with Pr-DuPHOS
1	R^1,R^2=H; R^3,R^4=Me	DIPAMP[16]	95	≥99
2	R^1,R^2,R^3=H; R^4=Me	SKEWPHOS[17]	99	≥99
3	R^1=Ph; R^2=H, R^3,R^4=Me	BICHEP[18]	97	≥99
4	R^1,R^4=Ph; R^2=H; R^3=Me	BINAP[19]	98	≥99
5	R^1=*i*-Pr; R^2=H; R^3,R^4=Me	PROPHOS[16]	81	≥99
6	R^1=*i*-Pr; R^2=H; R^3=Me; R^4=Ph	DIPAMP[20]	79	≥99

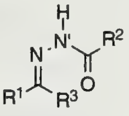
Since hydrogenation of (*E*)-*α*-enamides is facile, it follows that the analogous *β,β*-disubstituted and *β*-cyclic *α*-enamides are also hydrogenated with similar success. This class of substrates have been notoriously resistant to hydrogenation.[1] For example, for an *α*-enamide bearing two *β*–methyl groups (**10** R_1,R_2 = Me), the highest enantioselectivity previously reported was 55% using a DIPAMP-Rh catalyst.[16] However using the rhodium complexes of Me-BPE-Rh and Me-DuPHOS-Rh, ee's of 98.2% and 96% were obtained for the *R* and *S* configurations, respectively.[22] It is noteworthy that both *E* and *Z* *α*-enamides containing disparate *β*-substituents (e.g. R^1=Me, R^2=Et) can be hydrogenated with high enantioselectivities as well. The use of Me-BPE and Me-DuPHOS instead of their *n*-propyl analogs used in hydrogenating *β*-substituted *α*-enamides demonstrates the advantage of having easily changeable R groups on these ligand systems to match the steric demand of the substrate. On this particular substrate, for example, Rh-**8c** and Rh-**8d** produced valine with 45% and 14% ee, respectively.[22] To date, no other single asymmetric hydrogenation catalyst has been shown to provide consistently high ee's with such a wide variety of *α*-enamides.[9]

The *α*-enamides are useful starting materials in the production of a wide range of optically active amino acids, which themselves are precursors to a plethora of biologically active compounds.[23] For example, the tripeptide *N*-Boc-Met-Leu-Phe has been shown to be a good chemoattractant for human neutrophils.[24] Using **8d**, Burk and co-workers have synthesized a derivitive of this sequence, *N*-Boc-Met-Leu-o-Br-Phe-OMe which could, through Suzuki cross-couplings involving the aryl bromide, lead to many new derivatives,[25] which might also prove useful as chemotactive agonists and antagonists.

Hydrazone Hydrogenations

Unlike certain olefin and ketone reductions, the C=N bond has remained a challenge to hydrogenate asymmetrically with high enantioselectivity.[26] Recent advances have required either high pressures, high catalyst loadings, long reaction times, stoichiometric use of chiral starting materials, chiral auxillaries, or limited types of substrates.[27] The rhodium complex of the Et-DuPHOS ligand, however, has catalyzed reductions for a variety of *N*-acylhydrazones (**15**) with good ee's (51-97%) and excellent chemoselectivity

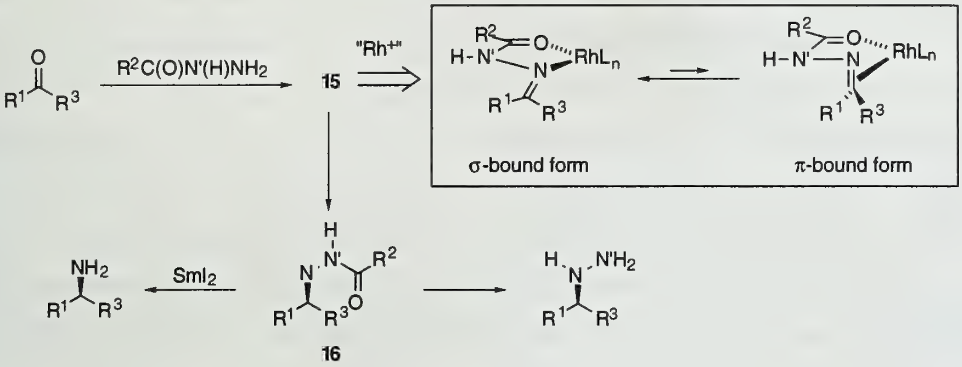
using mild conditions. These hydrazones are precursors for chiral hydrazines and chiral amines, which are important synthetic intermediates in drug design.[23]



15

The synthesis of these chiral hydrazines and amines begins with the condensation of a ketone and a carboxylic acid hydrazide to form the corresponding *N*-acylhydrazone (Scheme 3). Scarce information regarding intermediates in imine hydrogenations is available, although *N*-acylhydrazones σ-bound to Mn have been isolated and characterized.[28] However, recent evidence does suggest that the these σ-bound complexes may slip into a π-bound form prior to Rh-H insertion.[29] Both hydrolysis and cleavage proceed in high yield with little or no racemization.

Scheme 3



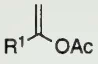
It is the introduction of the carbonyl oxygen three atoms removed from the C=N bond which allows the hydrogenation of these substrates to proceed. This promotes chelation to the metal center in a fashion similar to the α-enamides described earlier. Enantioselectivities for these substrates are highest when R^1 is an electron poor aryl group or ester, R^2 is an electron rich aryl group, and N' is secondary.[31] Chemoselectivities are excellent, with little or no reduction of ketone, aldehyde, ester, nitrile, carbon-halogen, nitro, alkene, or alkyne functionalities in the presence of **11**. Such high chemoselectivity in imine reductions is rare.[30]

When R^1=Ph and R^3=CO_2Me, hydrogenation of **15** with Rh-**7b** and Rh-**8b** produces arylglycines with an ee >91%.[27] These amino acids are not accessible through α-enamide hydrogenation. In addition, the imine precursors of α-hydrazino and α-amino phosphonates can be reduced in good ee's with the Et-DuPHOS catalyst. A prototypical substrate (R^1=Ph, R^3=$P(O)(OEt)_2$) was hydrogenated with 90% ee. These phosphonates are α-amino acid mimics which exert unique biological activity.[31]

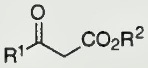
Burk's reductive amination of ketones might play a significant role in the production of two chiral α-amino phosphonate mimics of L-DOPA, an intermediate in the tyrosinase-mediated melanin biosynthesis pathway.[31]

Enol Acetate and β-Keto Ester Hydrogenation

Enol acetate (**17**) reductions are synthetically equivalent to keto group reductions, and therefore provide a useful route to chiral alcohols.[10] The scope of the reduction of these substrates with BPE and DuPHOS ligands has been investigated less thoroughly, but with encouraging results. When R^1 is electron-withdrawing, such as an ester or aryl group, ee's >90% are common with 100% conversion within 2-12 hours, in many cases outperforming previous attempts with electron-poor diphosphine catalysts.[10] For example, when R^1=CF_3, the best ee previously published was 77% , using a DIPAMP catalyst.[32] Use of Rh-**8a** provided the (*S*)-enantiomer with 94% ee.[10] Unlike the α-enamide and *N*-acylhydrazone reductions, substrates had to be sterically matched to the Me-BPE and Me-DuPHOS ligands to give the highest ee's.



17



18

Asymmetric hydrogenation of β-keto esters (**18**) lead to enantiomerically enriched β-hydroxy esters, which are valuable intermediates in synthetic and natural product chemistry.[11] In the absence of acid co-catalysts, recent progress using electron-poor catalysts has required either elevated temperatures (80-100 ˚C), high pressures (up to 103 atm), or long reaction times (up to 4 days).[11] Ruthenium complexes of **6c** on **14** (R^1=alkyl or cycloalkyl) provide the β-hydroxy ester with 100% conversion and excellent ee (95-99.4%) using mild conditions (35 ˚C, 4 atm H_2, $\leq$ 20 hrs).[11] The complex Rh-**5c** might be beneficial in synthesizing (*R*)-3(((allyloxy)carbonyl)oxy)decanoic acid via a mild β-keto ester reduction. This compound is a key intermediate in the synthesis of Rs-DPLA, an antagonist for endotoxin (LPS).[33]

CONCLUSIONS

The series of electron-rich bis(phosphalane) ligands developed by Burk possess rather unique properties which allow them to stereoselectively hydrogenate a variety of substrates with good to excellent ee's under mild conditions. In many cases, these ligands compare to or surpass the ee's of traditional electron-poor catalysts. These have potential applications to peptidomimetic research and development of LPS antagonists.

REFERENCES

(1) (a) Koenig, K.E.; Halpern, J. In *Asymmetric Synthesis*; Morrison, J.D., Ed.; Academic: New York, 1986; Vol. 5, Chapters 2,3. (b) Brunner, H. In *Topics in Stereochemistry*, Eliel, E.L., Wilen, S.H., Eds.; Interscience: New York, 1988; Vol. 18, p. 129.

(2) Whitesell, J.K. *Chem. Rev.* **1989**, *89*, 1581.

(3) Burk, M.J.; Feaster, J.E. *Tetrahedron: Asymmetry* **1991**, *2*, 569.

(4) Brunner, H.; Sievi, R. *J. Organomet. Chem.* **1987**, *328*, 71.

(5) Tani, K.; Suwa, K.; Tanigawa, E.; Ise, T.; Yamagata, T.; Tatsunu, X.; Otsuka, S. *J. Organomet. Chem.* **1989**, *370*, 203.

(6) Myashita, A.; Karina, H.; Shimamura, J.; Chiba, T.; Nagano, K.; Nohira, H.; Takaya, H. *Chem. Lett.* **1989**, 1849.

(7) Noyori, R.; Takaya, H.; Ohta, T. In *Catalytic Asymmetric Synthesis*; Ojima, J., Ed.; VCH: New York, 1993; Chapter 1.

(8) Burk, M.J.; Feaster, J.E.; Harlow, R.L. *Organometallics* **1990**, *9*, 2653.

(9) Burk, M.J.; Feaster, J.E.; Nugent, W.A.; Harlow, R.L. *J. Am. Chem. Soc.* **1993**, *115*, 10125.

(10) Burk, M.J. *J. Am. Chem. Soc.* **1991**, *113*, 8518.

(11) (a) Noyori, R.; Ohkuma, T.; Kitamura, M.; Takaya, H.; Sayo, N.; Kumobayashi, H.; Akutagawa, S. *J. Am. Chem. Soc.* **1987**, *109*, 5856. (b) Kitamura, M.; Ohkuma, T.; Inoue, S.; Sayo, N.; Kumobayashi, H.; Akutagawa, S.; Ohta, T.; Takaya, H.; Noyori, R. *J. Am. Chem. Soc.* **1988**, *110*, 629. (c) Kawano, H.; Ikariya, T.; Ishii, Y.; Sauri, M.; Yoshikawa, S.; Uchida, Y.; Kumobayashi, H. *J. Chem. Soc. Perkin Trans. I.* **1989**, 1571.

(12) Burk, M.J.; Harper, G.P.; Kalberg, C.S. *J. Am. Chem. Soc.* **1995**, *117*, 4423.

(13) For a similar example of a chiral phosphine breeder cycle, see: Fryzuk, M.D.; Bosnich, B. *J. Am. Chem. Soc.* **1978**, *18,* 5491.

(14) Burk, M.J.; Armstrong, S.K.; Brown, J.M. *Tetrahedron Lett.* **1993**, *34*, 879.

(15) Myashita, A.; Takaya, H.; Souchi, T.; Noyori, R. *Tetrahedron* **1984**, *40*, 1245.

(16) Scott, J.W.; Keith, D.D.; Nix, G.; Parrish, D.R.; Remington, S.; Roth, G.P.; Townsend, J.M.; Valentine, D.; Yang, R. *J. Org. Chem.* **1981**, *46*, 5086.

(17) Bosnich, B.; Roberts, N.K. In *Catalytic Aspects of Metal Phosphine Complexes*; American Chemical Society: Washington, DC, 1982; p. 337.

(18) Chiba, T.; Myashita, A.; Nohira, H.; Takaya, H. *Tetrahedron Lett.* **1991**, *32*, 4745.

(19) Ikariya, T.; Ishii, Y.; Kawano, H.; Arai, T.; Saburi, M.; Yoshikawa, S.; Akutagawa, S. *J. Chem Soc. Commun.* **1985**, 922.

(20) Vineyard, B.D.; Knowles, W.S.; Sabacky, M.J. *J. Mol. Catal.* **1983**, *19*, 159.

(21) For examples of this, see: (a) Fryzuk, M.D.; Bosnich, B. *J. Am. Chem. Soc.* **1977**, *99*, 6262. (b) Riley, D.P.; Shumate, R.E. *J. Org. Chem.* **1980**, *45*, 5187.

(22) Burk, M.J.; Gross, M.F.; Martinez, J.P. *J. Am. Chem. Soc.* **1995**, *117*, 9375.

(23) Burk, M.J.; Nugent, W.A.; Rajanbabu, T.V. *Science* **1995**, *117*, 4423.

(24) (a) Williams, T.; Snyderman, R.; Pike, M.C.; Lefkowitz, R.J. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 1204. (b) Schiffmann, E.; Corcoran, B.A.; Wahl, S.M. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72,* 1059. (c) Toniolo, C.; Crisma, M.; Moretto, V.; Freer, R.J.; Becker, E.L.; *Biochim. Biophys. Acta.* **1990**, *1034*, 67.

(25) Burk, M.J.; Lee, J.R.; Martinez, J.P. *J. Am Chem. Soc.* **1994**, *116*, 10847.

(26) Burk, M.J.; Feaster, J.E. *J. Am. Chem. Soc.* **1992**, *114*, 6266.

(27) Burk, M.J.; Martinez, J.P.; Feaster, J.E.; Cosford, N. *Tetrahedron* **1994**, *50*, 4399.

(28) (a) Pelizzi, C.; Pelizzi, G.; Tarasconi, P. *J. Chem. Soc., Dalton Trans.* **1985**, 215. (b) Liufang, W.; Ying, Z.; Zhengyin, Y.; Jigui, W.; Qi, W. *Polyhedron* **1991**, *10*, 2477.

(29) Fryzuk, M.D.; Piers, W.E. *Organometallics* **1990**, *9*, 986.

(30) For a recent example of similarly high chemoselectivity, see: Ng Cheong Chan, Y.; Meyer, D.; Osborn, J.A. *J. Chem. Soc., Chem Commun.* **1990**, 869.

(31) Lecjczak, B.; Kafarski, P.; Makowiecka, E. *Biochem. J.* **1986**, *242*, 81.

(32) Koenig, K.E.; Bachman, G.L.; Vineyard, B.D. *J. Org. Chem.* **1980**, *45,* 2362.

(33) Delude, R.L.; Savedra, R.; Zhao, H.; Theringer, R.; Yamamoto, S.; Fenton, M.J.; Golenback, D.T. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 9288.

PREPARATION OF FUNCTIONALIZED DIALKYLZINCS AND THEIR CATALYTIC ASYMMETRIC ADDITIONS TO ALDEHYDES

Reported by Shaun Stauffer November 16, 1995

INTRODUCTION

In the last decade organozinc reagents, particularly alkylzinc halides (RZnX), have become versatile synthetic building blocks.[1a-c] Their chemoselective nature permits the rapid preparation of highly functionalized molecules ordinarily not accessible using conventional magnesium and lithium reagents.

Dialkylzincs (R_2Zn) have also attracted a considerable amount of attention, since they are the first successful organometallics to add stereoselectively to aldehydes in a catalytic manner.[2a] However, in contrast to alkylzinc halides, general methods to prepare dialkylzincs have been lacking until recently. Consequently, this has limited the scope of such catalytic asymmetric additions to relatively simple, unfunctionalized dialkylzincs (i.e. Et_2Zn).[2a]

It is now feasible to prepare dialkylzincs containing a broad range of functionality, including esters, halides, acrylates, protected amines, silylethers, and silylenol ethers. These groups are well tolerated in a catalytic enantioselective aldehyde addition and provide a route to versatile polyfunctional alcohols in enantioenriched form.[1d] This review will focus on recent methods used for the preparation of functionalized dialkylzincs and their applications in catalytic asymmetric additions to aldehydes.

BACKGROUND

Reactivity and Structure of R_2Zn

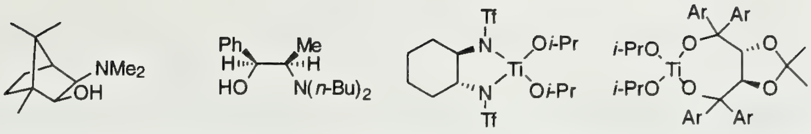
Dialkylzincs have a moderately strong Zn-C bond energy of ca. 42 kcal/mol. Their high degree of covalency (85% for Me_2Zn) and linear *sp*-hybridized geometry render them extremely poor nucleophiles by themselves.[1b] However, this low reactivity permits the tolerance of a wide range of functional groups.

Upon complexation with a Lewis base, coordination at Zn changes from linear to tetrahedral. This causes an increase in Zn-C bond length, increasing the potential for nucleophilic addition (nucleophilic catalysis). In addition, electrophilic catalysis is possible when an effective Lewis acid coordinates with the carbonyl oxygen of the aldehyde.[2a,b]

Catalysts and General Mechanisms

Due to the enormous interest in this catalytic C-C bond forming reaction, a plethora of catalysts have appeared. The β-aminoalcohol ligands were the first such catalysts.[2,3] The camphor derived (-)-3-*exo*-(dimethylamino)isoborneol catalyst (**1**)[2a-d] developed by Noyori and the (-)-(1*S*,2*R*)-*N*,*N*-dibutylnorephedrine catalyst (**2**)[2a,e] developled by Soai (Figure 1) are two of the most successful. Both provide chiral alcohols in high enantiomeric excess (ee) using 2-6 mol% loading of the chiral ligand.

Copyright © 1995 by Shaun Stauffer



1 2 3 4

Ar = 2-naphthyl

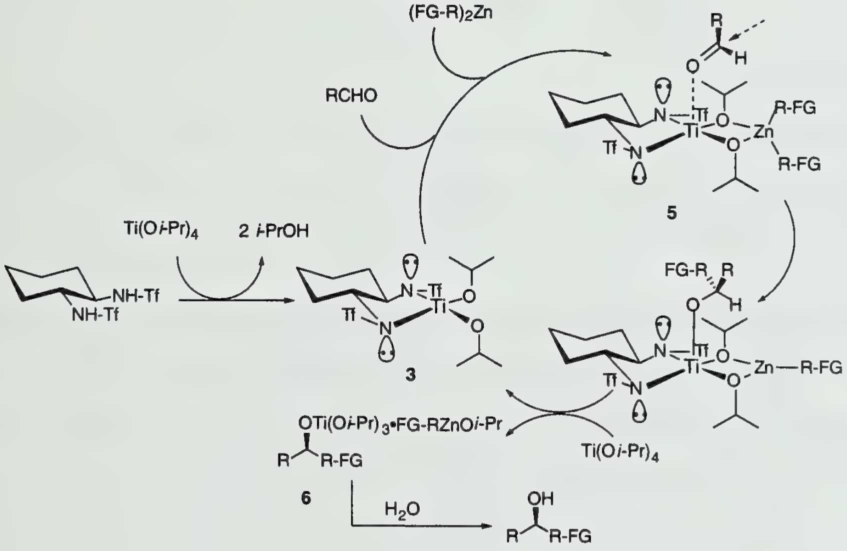
Figure 1. Selected Catalysts used for R_2Zn Addition to Aldehydes.

The mechanism and catalytic cycle for the aminoalcohol catalyzed reaction is now well understood.[1b,2a,4a,b] The species responsible for catalysis is a pre-formed ligand-zincate complex which coordinates both to the aldehyde and a second R_2Zn. An R-group is then internally transferred stereoselectively. Both a chair-like six membered transition state[2e] and a bridged alkyl 4/4 transition state[2b,d] have been proposed. Recent ab initio studies by Noyori and Yomakawa have shown that the 4/4 transition state structure is more stable by 4-7 kcal/mol.[4c]

C_2-Symmetrical chiral titanate ligands have most recently been reported (**3** and **4**, Figure 1) and appear to be superior catalysts compared to the β-aminoalcohol ligands.[5a] The most general Ti-catalyst, **3**, was reported in 1989 by Yoshioka, Ohno, and coworkers.[5b-d] It uses the rather slender *trans*-(1*R*, 2*R*)-bis(trifluoromethanesulfonamido)cyclohexane as a chiral ligand. Reaction times for the addition of dialkylzinc reagents are relatively short compared to the β-aminoalcohols (2 h vs. 16 h) and excellent turnovers are possible (0.05 mol%, 2000). In addition to hexane and toluene, diethyl ether may be used as a solvent, thus allowing a convenient method towards unfunctionalized R_2Zn from common Grignard reagents.[5e] Extensive applications using functionalized dialkylzincs ($(FG\text{-}R)_2Zn$, FG='functional group') have been conducted using this catalyst;[1a,6] however, mechanistic studies are still desired.[5a-c]

It is known that a ligand acceleration effect[7] is responsible for the desired catalytic efficacy; hence, the need for stoichiometric quantities of the cocatalyst, $Ti(O\textit{i}\text{-}Pr)_4$.[5a] The active species that delivers the alkyl group to the carbonyl compound is still unclear. A proposed mechanism and catalytic cycle which rationalizes the origin of *si* selectivity is shown in Scheme 1.[8]

Scheme 1



The *pseudo*-equatorial trifluoromethanesulfonyl groups are thought to direct the position of the two isopropoxy ligands (**3**). Upon complexation to form the bimetallic complex, **5**, only the *si* face of the aldehyde is accessible to internal addition.[8,9a] The $Ti(O\textit{i}\text{-}Pr)_4$ removes the product alkoxide from the chiral titanium center through ligand exchange resulting in the formation of a mixed complex, **6**, regenerating the catalyst.

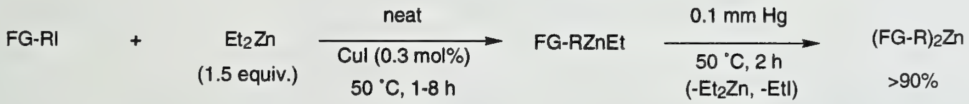
Seebach and coworkers have demonstrated that bulky titanate complexes, such as **4**, can also catalyze the addition of dialkylzincs to aldehydes with excellent ee's.[9b] Stoichiometric amounts of titanium alkoxide cocatalysts and 10-20 mol% loading of the chiral ligand are required in order to achieve acceptable rates. In contrast to **3**, the titanium complex **4** accelerates the reaction without electron withdrawing groups. It is believed that bulky tartrate-derived ligands hinder self-aggregation, thus allowing R_2Zn complexation to be more rapid, affecting catalysis.[5a] Unfortunately, functionalized and less reactive dialkylzincs do not appear to work well with these catalysts, often resulting in low ee.[9c]

PREPARATION AND APPLICATIONS OF $(FG\text{-}R)_2Zn$

The Iodine-Zinc Exchange Method

For simple unfunctionalized dialkylzincs, either oxidative addition or transmetallation with a Grignard reagent can provide the dialkylzinc.[1a,b] A route towards functionalized dialkylzincs was realized in 1992, when Knochel and coworkers found that primary alkyl iodides undergo an iodine-zinc exchange with Et_2Zn to form $(FG\text{-}R)_2Zn$.[10a] The reaction was later greatly enhanced by adding catalytic amounts of CuI, thus eliminating the need for large excess amounts of Et_2Zn (Scheme 2). Reaction rates increased two-fold and conversions over 95% were common for most primary iodides, even on a large scale. Mechanistic studies have not been conducted to explain the origin of Cu(I) catalysis, but a radical chain reaction has tentatively been proposed.[10b]

Scheme 2

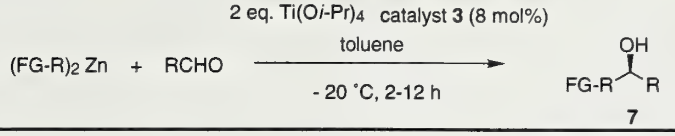


$$\text{FG-RI} + \underset{(1.5\text{ equiv.})}{Et_2Zn} \xrightarrow[\substack{\text{CuI (0.3 mol\%)} \\ 50\ ^\circ\text{C, 1-8 h}}]{\text{neat}} \text{FG-RZnEt} \xrightarrow[\substack{50\ ^\circ\text{C, 2 h} \\ (\text{-}Et_2Zn,\ \text{-}EtI)}]{\text{0.1 mm Hg}} \underset{>90\%}{(\text{FG-R})_2Zn}$$

Dialkylzincs containing esters, chlorides, protected amines, and silylethers have been prepared in this manner and used in situ in the enantioselective preparation of secondary alcohols using catalyst **3**.[1a,10a] Excellent ee's are obtained for a variety of unfunctionalized aliphatic and aromatic aldehydes, including α–branched aldehydes (entry e Table 1).

The position and nature of functional groups within both the zinc reagent and the aldehyde can have dramatic effects on ee. Organozincs containing heteroatoms can form chelates which tend to reduce enantioselectivity and chemical yield. For example, excellent ee's are possible for esters separated four or five carbons from the metal center (entries b and e, Table 1), but for reagents with a shorter alkyl chain, such as bis(acetoxypropyl)zinc reagents (entry c), the ee's are compromised.[10a] The use of a bulkier pivaloyl ester (entry d) reduces chelation at the metal center raising enantioselectivities to greater than 90%.

Table 1. Additions of $(FG\text{-}R)_2Zn$ to Simple Unfunctionalized Aldehydes

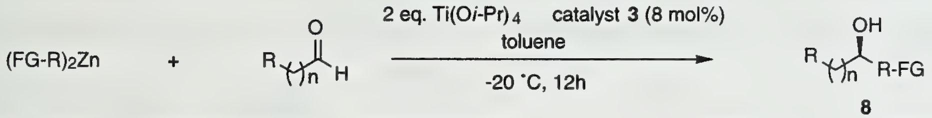


entry	FG-R	RCHO	% Yield	% ee
a	$Cl(CH_2)_4$	PhCHO	95	93
b	$AcO(CH_2)_4$	PhCHO	72	92
c	$AcO(CH_2)_3$	PhCHO	75	86
d	$PivO(CH_2)_3$	PhCHO	90	92
e	$AcO(CH_2)_5$	*c*-HexCHO	83	97

Functionalized aliphatic aldehydes have also been used as substrates with these $(FG\text{-}R)_2Zn$ reagents (Table 2). Enantiopurities from good to excellent have been obtained for *α*–,[10b] *β*–,[10c] and *γ*-silyloxy aldehydes (entries a-d),[10d] *β*-stannyl aldehydes (entry e),[10e] *β*-silylaldehydes, and *β*-benzyloxy aldehydes.[10d] When a stereogenic center is already present in the aldehyde (either *β* or *γ* silyloxy), the stereochemical outcome for the addition product is strictly controlled by the chirality of the catalyst.[6,10d,e] This allows, for example, complete control of stereochemistry among a set of possible diol diastereomers.

For silyloxy aldehydes, the choice of triorganosilyl moiety is crucial in order to prevent chelation at the titanium center. In general, the protecting group selected depends on the chain length and reactivity of the diorganozinc. For example, less reactive zinc reagents require bulkier protecting groups on the aldehyde substrate in order to obtain satisfactory enantiopurity; however, significant variations in ligand exchange kinetics can occur resulting in lower chemical yields (compare entries b and c Table 2).[8,10d]

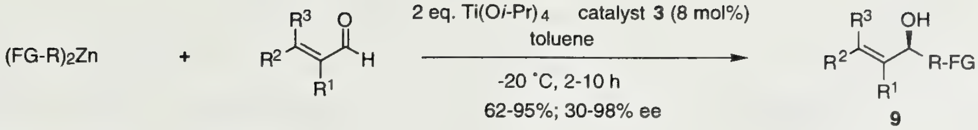
Table 2. Additions of $(FG\text{-}R)_2Zn$ to Functionalized Aldehydes



entry	FG-R	R	n	%Yield	% ee
a	$PivO(CH_2)_5$	$\text{-OSi}(i\text{-Pr})_3$	1	74	90
b	$PivO(CH_2)_3$	$\text{-OSi}(i\text{-Pr})_3$	2	55	40
c	$PivO(CH_2)_3$	$\text{-OSiPh}_2t\text{-Bu}$	2	35	98
d	$PivO(CH_2)_3$	$\text{-OSi}(i\text{-Pr})_3$	3	62	96
e	Octyl	-SnBu_3	2	82	93

Simple unfunctionalized *α,β*-unsaturated aldehydes are suitable substrates, affording highly enantioenriched allylic alcohols (**9**, Scheme 3) in 80-98% ee.[11a] Dialkylzinc reagents containing esters, chlorides, alkyl groups, and protected amines are well tolerated affording polyfunctional chiral alcohols.

Scheme 3



The presence of an alkyl group or halogen at the R^1 of the aldehyde dramatically enhances enantioselectivity. This forces the carbonyl compound to adopt a *syn* conformation, which locates the double bond closer to the stereogenic metal center upon complexation, thus allowing better steric differentiation between the two carbonyl faces. However, in some cases, substitution at R^1 can be avoided with sterically unhindered aldehydes by using a bulkier titanium alkoxide cocatalyst.[11b] For (*Z*)-α,β-unsaturated aldehydes, where R^3 is other than hydrogen, the ee's are moderate to poor, further supporting the argument of a highly coordinated intermediate at the carbonyl oxygen.[11a]

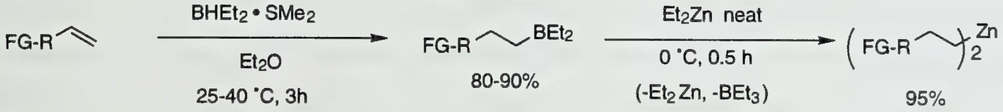
Functionalized (*E*)-α,β-unsaturated aldehydes, including β-silyl,[8] β-stannyl,[8,10b] β'-silyloxy, and γ-silyloxy, have been utilized as substrates.[10c] Using the iodine-zinc exchange method, organozincs containing esters, chlorides, and alkyl groups are all well tolerated and add with excellent stereoselectivity affording allylic alcohols of type **9**. Dialkylzincs containing protected amines provide lower enantioselectivities, approaching 86%. In contrast to their unfunctionalized counterparts, functionalized unsaturated aldehyde substrates do not require substitution at R^1 in order to achieve high ee. For chiral substrates, where β' or γ are triorganosilyloxy substituents, the stereochemical outcome is controlled solely by the chirality of the catalyst, allowing the preparation of all possible 1,3 and 1,4 unsaturated diol stereoisomers. An interesting inverse temperature effect was observed when β-silyl[10c] and γ-silyloxy[10d] aldehydes were used with less reactive zinc reagents. For instance, only 80% ee was observed for the type **9** product when bis-(3-pivaloxypropyl)zinc was added to (*E*)-3-(trimethylsilyl)-2-propenal at -40 °C; however, at -15 °C, a 90% ee was attained. The positive ee enhancement erodes above -5 °C. Similarly, stereochemical and chemical yields were dependent on the triorganosilyloxy moiety choosen.[10c]

The Boron-Zinc Exchange Method

The boron-zinc exchange reaction now constitutes one of the most general methods to prepare dialkylzincs.[6a] Due the fact that alkyl iodides are usually obtained from either an olefin or alcohol, the boron-zinc exchange reaction provides a more attractive, "halide free" route.

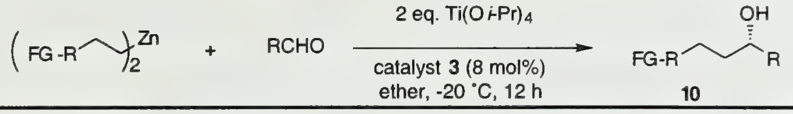
Using BEt_2H, prepared from BEt_3 and BH_3, various functionalized dialkylzincs can be prepared (Scheme 4) and then used in a catalytic asymmetric addition to aldehydes. Functional groups tolerated include silylethers, acrylates, bromides, iodides[13a], and silylenol ethers.[12]

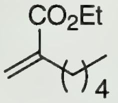
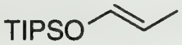
Scheme 4



Starting from a terminal alkene treatment with one equivalent of BEt_2H affords a mixed diethyl(alkyl)borane in excellent yield. Subsequent transmetallation with Et_2Zn occurs within 30 minutes at 0 °C. The driving force for the reaction appears to be the removal of the more volatile Et_3B via distillation. The resulting zinc reagents are suitable donors in a one-pot synthesis of optically active secondary alcohols of type **10** using catalyst **3** (Table 3).[6,13]

Table 3. Additions of $(FG\text{-}R)_2Zn$ to Simple Unfunctionalized Aldehydes.



entry	$FG\text{-}R^1$	R^2	%Yield	% ee
a	$PivO(CH_2)_3$	Ph	70	93
b	$t\text{-}Bu(CH_3)_2SiO(CH_2)_4$	Ph	82	92
c		C_5H_{11}	72	95
d	$Br(CH_2)_6$	C_5H_{11}	77	90
e		Ph	82	93

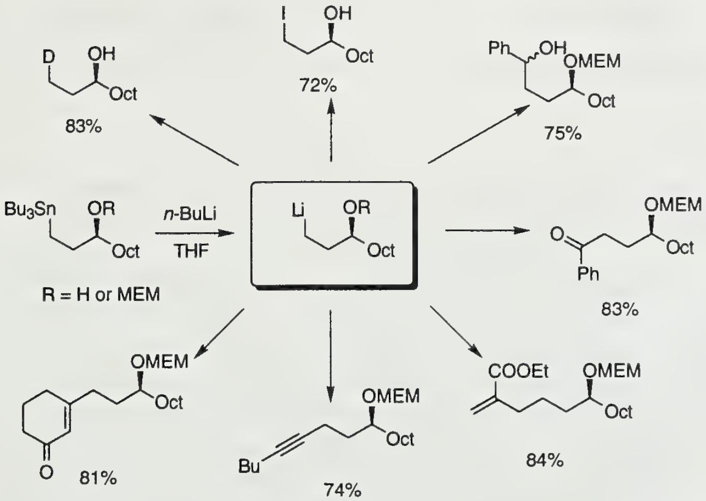
Simple, unfunctionalized aliphatic, aromatic, and unsaturated aldehydes are all acceptable substrates affording the *si* addition product in high enantiopurity. The sensitive acrylate functionality (entry c) is well tolerated and provides a versatile route towards enantioenriched unsaturated hydroxyesters. Silyl ethers and silylenol ethers (for example entries b and e, respectively) both contain sensitive oxygen functionalities that are also well tolerated. Unhindered secondary dialkylzincs, which are difficult to make by other means, can be prepared using the boron-zinc exchange method although longer reaction times are required.[13a] Functionalized aldehydes have yet to be investigated as substrates.

SYNTHETIC UTILITY

Chiral secondary alcohols derived from the catalytic asymmetric addition of functionalized diorganozincs to aldehydes are highly versatile intermediates. For example, α-silyloxy aldehydes (8a Table 2) provide an alternative approach towards chiral 1,2 diols and epoxides.[10c] The β-silyloxy aldehydes (8b and 8c Table 2) have been demonstrated to afford chiral aldol-like products, which can be elaborated further in a catalytic dialkylzinc addition to afford either *syn* or *anti*-1,3 diols.[10d] The γ-silyloxy aldehydes (8d Table 2), despite being potential chelators, have successfully been used to prepare C_2 -symmetrical 1,4-diols as well as *syn*-1,4-diols in high diastereomeric excess.[10e]

β-Stannyl aliphatic aldehydes (8e Table 2) provide an excellent route towards protected chiral 1,3-hydroxystannyl synthons. Upon transmetallation of the stannane to the corresponding lithium compound, various electrophiles can be added to afford enantioenriched molecules of interest (Scheme 5).

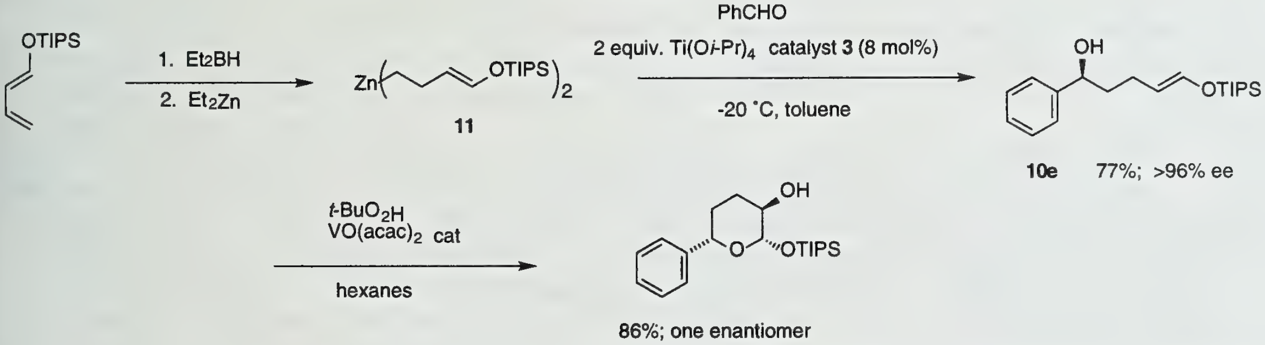
Scheme 5



In addition, chiral γ-alkoxyenones can be obtained from *β*-stannyl unsaturated aldehydes using a Stille acylation reaction after enantioselective addition.[8] These chiral intermediates are useful Michael acceptors for diastereoselective conjugate additions of organocuprates.[14]

The enantioenriched bis-homoallylic alcohol, compound **10e**, obtained from the addition of **11** and benzaldehyde, has been utilized in the preparation of a single enantiomer tetrahydropyran derivative after successful epoxidation and ring closure (Scheme 6).[13b]

Scheme 6



The ability to have bromides present (10d Table 3) has permitted a rapid synthesis of *(S)*-10-nonacosanol (ginnol) in three steps affording product in 61% overall yield (>92% ee).[13a] In one application starting from an alkyne, the boron-zinc exchange method was elegantly exploited in a macrocyclization to afford *(R)*-(-)-muscone using catalyst **1**.[15] More recently, the synthetic utility of functionalized dialkylzincs has been demonstrated in the total syntheses of both the antitumor antibiotic (-)-methylenolactocin,[16] and the jamonoid (+)-methyl epijasmonate.[17]

CONCLUSION

The iodine-zinc and boron-zinc exchange reactions permit the mild and rapid preparation of dialkylzincs containing a broad range of functionality. These reagents are versatile organometallics which successfully add to aldehydes in a catalytic asymmetric addition reaction. The scope of this reaction has yet

to be fully exploited; however, its chemoselective nature and current success clearly demonstrate the versatility of this catalytic asymmetric carbon-carbon bond forming reaction. This methodology now provides a valuable alternative to the asymmetric reduction of functionalized ketones.

REFERENCES

1a) Singer, P.; Knochel, P. *Chem. Rev.* **1993**, *93*, 2117. b) Knochel, P. *COS*, vol. 2, **1991**, 211. c) *Organometallics in Synthesis: A Manual.* Schlosser, M., Ed.; J. Wiley & Sons, 1994. d) Nugent, W.; RajanBabu, T. V.; Burk, M. *Science* **1993**, *259*, 479.

2a) Soai, K; Niwa, S. *Chem. Rev.* **1992**, *92*, 833. b) Noyori, R.; Kitamura, M. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 49. c) Noyori, R.; Kawai, K.; Suga, S.; Kitamura, M. *J. Am. Chem. Soc.* **1986**, *108*, 6071. d) Noyori, R.; Suga, S.; Okada, S.; Kitamura, M. *J. Am. Chem. Soc.* **1989**, *111*, 4028. e) Soai, K.; Yokoyama, S.; Hayasaka, T. *J. Org. Chem.* **1991**, *56*, 4264.

3a) Fuji, K.; Tanaka, K.; Miyamoto, H. *Chem. Pharm. Bull.* **1993**, *41*, 1557. b) Wiliams, M. J.; Frost, C.; Allen, J. *Tetrahedron: Asymmetry* **1993**, *4*, 649. c) Williams, M. J.; Allen, J. V. *Tetrahedron: Asymmetry* **1994**, *5*, 277. d) Luche, J. L.; Einhorn, C.; Delair, P. *Tetrahedron* **1995**, *51*, 165. e) Soai, K.; Ookawa, A.; Kaba, T., Ogawa, K. *J. Am. Chem. Soc.* **1987**, *109*, 7111. f) Hoshino, O.; Shimamoto, M.; Fujita, K.; Ishizaki, M. *Tetrahedron: Asymmetry* **1994**, *5*, 411. g) Schlogl, K.; Weissensteiner, W.; Widhalm, M.; Wally, H. *Tetrahedron: Asymmetry* **1993**, *4*, 285. h) Uemura, M.; Miyake, R.; Nakayama, K.; Shiro, M.; Hayashi, Y. *J. Org. Chem.* **1993**, *58*, 1238. i) Carreno, M. C.; Ruano, J.; Maestro, M.; Cabrejas, L. M. *Tetrahedron: Asymmetry*, **1993**, *4*, 727. j) Bolm, C.; Muller, J. *Tetrahedron* **1994**, *50*, 4355. k) Kang, J.; Kim, D. S.; Kim, J. *Synlett* **1994**, 842. l) Martens, J.; Mehler, T. *Tetrahedron: Asymmetry*, **1994**, *5*, 207. m) Caze, C.; Moualij, N. *Eur. Polym. J.* **1995**, *31*, 193. n) Soai, K.; Watanabe, M. *J. Chem. Soc. Perkin Trans. 1*, **1994**, 837.

4a) Noyori, R.; Kitamura, M.; Suga, S.; Niwa, M.; Zhai, Z.; Suga, H. *J. Phys. Chem.* **1994**, *98*, 12776. b) Noyori, R.; Niwa, M.; Suga, S.; Kitamura, M. *J. Am. Chem. Soc.* **1995**, *117*, 4832. c) Noyori, R.; Yamakawa, M. *J. Am. Chem. Soc.* **1995**, *117*, 6327.

5a) Duthaler, R.; Hafner, A. *Chem. Rev.* **1992**, *92*, 807. b) Yoshioka, M.; Kawakita, T.; Ohno, M. *Tetrahedron Lett.* **1989**, *30*, 1657. c) Yoshioka, M.; Ohno, M.; Kobayashi, S.; Takahashi, H.; Kawakita, T. 1*TetrahedronLett.* **1989**, *30*, 7095. d) Takahashi, H.; Kawakita, T.; Ohno, M.; Yoshioka, M.; Kobayashi, S. *Tetrahedron* **1992**, *48*, 5691.

6) Knochel, P. *Synlett* **1995**, 393.

7) Sharpless, K. B.; Bolm, C.; Berrisford, D. J. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1059.

8) Knochel, P.; Stadtmuller, H.; Chavant, P.-Y.; Ostwald, R. *J. Org. Chem.* **1994**, *59*, 4143.

9a) Seebach, D.; Petter, W.; Plattner, D. A.; Beck, A. K.; Wang, Y. M.; Hunziker, D. *Helv. Chim. Acta* **1992**, *75*, 2171. b) Seebach, D.; Beck, A.; Schmidt, B.; Wang, Y. *Tetrahedron* **1994**, *50*, 4363. c) Seebach, D.; Bussche-Hunnefeld, J. *Tetrahedron* **1992**, *48*, 5719. d) Seebach, D; Schmidt, B Angew. *Chem. Int. Ed. Engl.* **1991**, *30*, 99. e) Seebach, D.; Schmidt, B. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1321.

10a) Knochel, P.; Sidduri, A.; Rozema, M. *J. Org. Chem.* **1992**, *57*, 1956. b) Knochel, P.; Eisenberg, C. *J. Org. Chem.* **1994**, *59*, 3760. c) Knochel, P.; Vettel, S. *Tetrahedron Lett.* **1993**, *34*, 5881. d) Knochel, P.; Vettel, S. *Tetrahedron Lett.* **1994**, *35*, 5849. e) Knochel, P.; Brieden, W.; Ostwald, R. *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 582.

11a) Knochel, P.; Rozema, M.; Eisenberg, C.; Lutjens, H.; Ostwald, R.; Belyk, K. *Tetrahedron Lett.* **1993**, *34*, 3115. b) Knochel, P.; Nowotny, S.; Vettel, S. *Tetrahedron Lett.* **1994**, *35*, 4539.

12a) Srebnik, M. *Tetrahedron Lett.* **1991**, *32*, 2449. b) Knochel, P.; Waas, J.; Langer, F. *Tetrahedron Lett.* **1993**, *34*, 5261. c) Knochel, P.; Langer, F.; Devasagayaraj, A.; Chavant, P.-Y. *Synlett* **1994**, 410.

13a) Knochel, P.; Schwink, L. *Tetrahedron Lett.* **1994**, *35*, 9007. b) Knochel, P.; Devasagayaraj, A.; Schwink, L. *J. Org. Chem.* **1995**, *60*, 3311.

14a) Yamamoto, Y.; Chounan, Y.; Nishii, S., Ibuka, T.; Kitahara, H. *J. Am. Chem. Soc.* **1992**, *114*, 7652. b) Normant, J.-F.; Alexakis, A.; Marek, I. *Tetrahedron* **1991**, *32*, 6337.

15) Radinov, R.; Oppolzer, W. *J. Am. Chem. Soc.* **1993**, *115*, 1593.

16) Knochel, P.; Vaupel, A. *Tetrehedron Lett.* **1995**, *36*, 231.

17) Knochel, P.; Stadtmuller, H. *Synlett* **1995**, 463.

LIVING FREE RADICAL POLYMERIZATION

Reported by Peggy-Jean Prest November 27, 1995

INTRODUCTION

Most of the industrially important polymers today are synthesized using radical chemistry.[1,2] Although the chain length distribution in radical polymerizations is unpredictable due to uncontrollable termination by disproportionation or combination, radical additions proceed rapidly and can be applied to a wide range of monomers. The understanding of radical reactions involving small molecules has recently advanced,[3] and the ability to further control radical reactivity has been paralleled by new discoveries in the field of radical polymerizations. The method currently being developed in order to eliminate termination is the living free radical polymerization reaction.[4-7] In living anionic, cationic, and ROMP polymerizations, termination is controlled and nearly uniform chain lengths are obtained. However, these processes are not amenable to large-scale applications as the reactions apply to select monomers and are highly sensitive towards impurities.[1,2] Therefore, the development of a living radical polymerization process for the production of highly monodisperse polymers has significant industrial value.

The term 'living polymerization' is defined as a polymerization in which the chain ends retain the ability to react with monomers throughout a reaction.[1,2,6,8] In ideal living systems there are an equal number of initiators as growing chains, and the chains grow to nearly identical lengths since the rate of initiation is faster than the rate of propagation, propagation is constant, and no termination occurs. In contrast to chain growth polymers, which terminate after the initial monomers have been consumed, the chains of living systems resume growth whenever more monomer is added to the reaction. To determine experimentally if a polymerization is living, the polydispersity and rate of monomer consumption must be analyzed.[1] Polydispersity, (PDI), defined as the weight average molecular weight, M_w, divided by the number average molecular weight, M_n, can be measured by gel permeation chromatography (GPC). The value of M_w/M_n (PDI) is unity for a perfectly monodisperse polymer and is larger than one when there is a distribution of chain lengths. A living system produces nearly monodisperse polymers. In a living polymerization, the molecular weight of the growing polymer is directly proportional to the percent of monomer converted to polymer, whereas in a nonliving polymerization high molecular weight materials are formed in the initial stages of the reaction.[6] Ideal living polymerization is a rare process, as the active chain ends of most systems are stabilized by counterions or solvent.[8] Therefore, in order to control chain growth in a living free radical system, the propagating radical centers must be protected from termination with reversible capping agents.

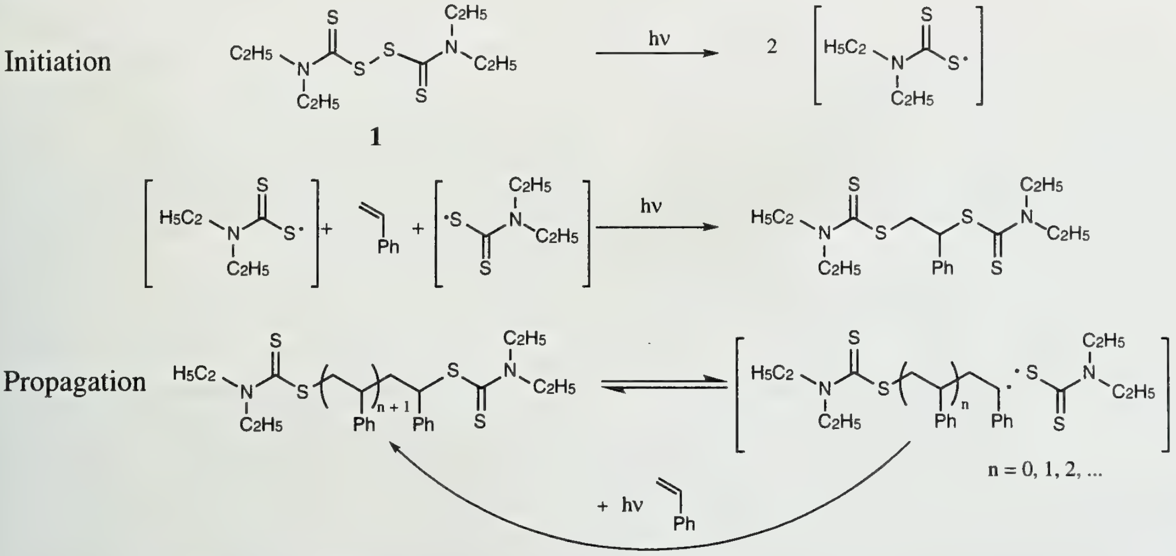
BACKGROUND

In 1982, Otsu designed a class of terminal trapping agents to bind reversibly to propagating radicals.[9,10] These agents, referred to as initiator-transfer-agent-terminators, and commonly shortened to 'iniferters', serve as both photochemical initiators and reversible terminators of the chain. For example, the

Copyright © 1995 by Peggy-Jean Prest

polymerization of styrene using the 'iniferter' tetraethylthiuramidesulfide **1**, (Scheme I) involves the following steps: 1) photolytic cleavage of the dithiocarbamate bond, 2) addition of styrene and 3) reversible chain termination using the remaining dithiocarbamate radical as a trapping agent. The usefulness of this particular system is limited by 1) the slow rate of propagation due to the high carbon-sulfur bond strength and 2) the ability of the iniferter fragment to initiate new chains, which ultimately leads to broadly disperse chains.[11] Although the 'iniferter' method was not as successful as initially hoped, the experimental work carried out laid the groundwork for the development of other trapping agents, suggesting that the idea of developing a living free radical polymerization was viable.

Scheme I



Other radical trapping agents, such as the stable nitroxide radical, 2,2,6,6-tetramethyl-piperdine-1-oxy (TEMPO, **3** in Scheme II) have been studied.[12-14] TEMPO reacts at nearly diffusion controlled rates to trap carbon radicals and does not react with oxygen-centered radicals.[15] When benzoyl peroxide initiators and styrene monomers are mixed with TEMPO, TEMPO traps the first carbon radical that is formed, compound **4** in Scheme II. The bond formed between TEMPO and styrene in adduct **4** is stable at temperatures below 100 °C and no polymerization occurs.

The relative strength of the bond connecting 1-substituted ethylbenzenes to a variety of different stable free radicals has been calculated using the semiempirical methods AM1 and PM3.[16] Bond dissociation enthalpies for a selective listing of stable free radicals are shown in Table I. Here, benzoyl radicals ($PhCO_2\bullet$) form stronger bonds with 1-substituted ethylbenzene radicals than TEMPO. These enthalpy values can be used as a guideline for choosing a stable free radical (SFR) with the potential to reversibly terminate chain growth.

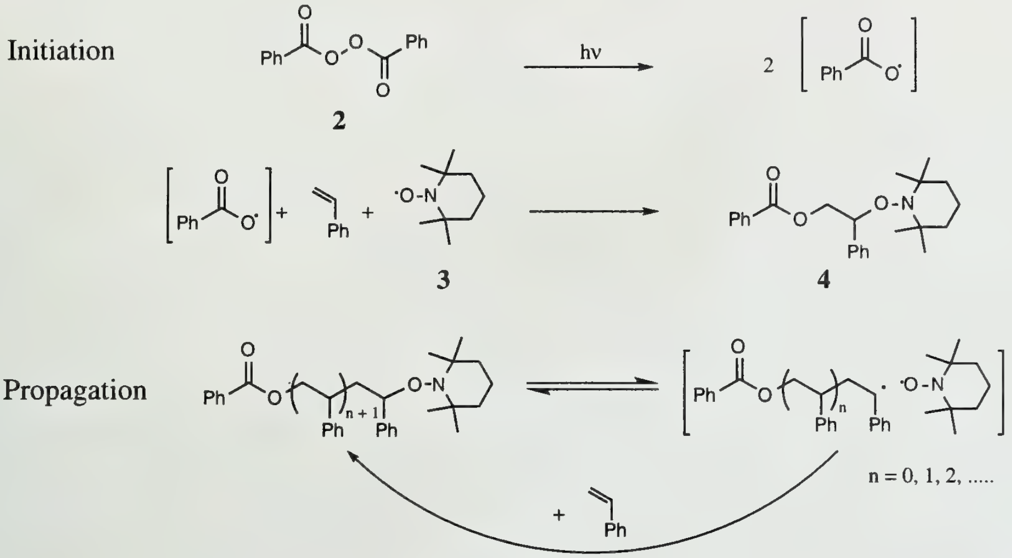
Table I. Calculated Bond Enthalpies (kcal/mol) for 1-Substituted Ethylbenzene and Radical Species.

Radical	AM1	PM3
$PhCO_2\bullet$	59	63
$Me_2NCS_2\bullet$	38	39
TEMPO	22	26

ORGANIC LIVING FREE RADICAL POLYMERIZATIONS

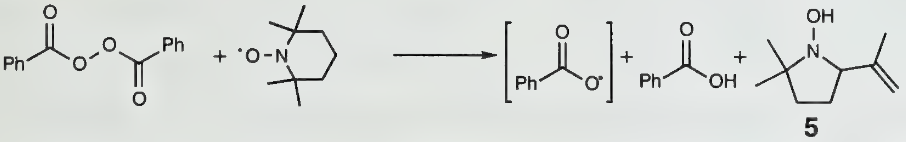
Above 100 °C, a radical polymerization of styrene using TEMPO as the reversible terminating agent and benzoyl peroxide (BPO **2** in Scheme II), as the initiator has been determined to be living.[4,17,18] At elevated temperatures, 1) the rate of initiation by thermal homolysis exceeds that by TEMPO-promoted dissociation,[19] 2) initiation occurs almost instantaneously,[19,20] 3) the TEMPO-styrene bond is labile towards homolysis,[16] and 4) autopolymerization of styrene increases.[18,21,22] These living polymers grow in a stepwise manner after rapid initiation, as illustrated in Scheme II, to yield high molecular weight chains with narrow polydispersities (PDI = 1.26). Upon cooling, propagation stops due to insufficient energy to break the TEMPO-styrene bond. Reheating the polymer re-initiates the propagation process.[4]

Scheme II



For all the chains to begin growth simultaneously, benzoyloxy radicals must form and initiate within a short time span. At high temperatures, BPO **2**, has a short half-life, and dissociation *via* the thermal or TEMPO-promoted mechanism occurs quickly.[19,20] In thermal dissociation, two benzoyloxy radicals are formed, whereas the TEMPO promoted dissociation produces different products: 5-isoprenyl-2,2-dimethyl-pyrrolidin-1-ol, **5**, benzoic acid, and one benzoyloxy radical as shown in Scheme III. Although these processes compete at room temperature, when subjected to temperatures above 100 °C, thermal dissociation occurs almost instantly. Therefore, this system fulfills the living criteria, i.e. simultaneous initiation of polymer chains.

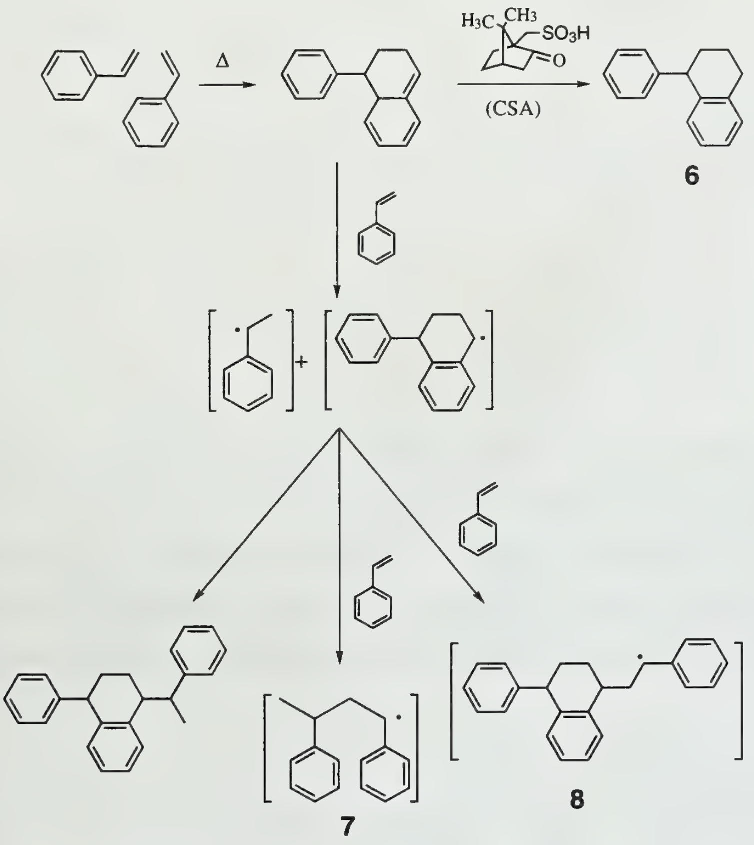
Scheme III



At elevated temperatures, the autopolymerization of styrene, a polymerization that is both initiated and propagated by styrene itself, starts to compete with the BPO-initiated living polymerization.[18,21,22] To reduce autopolymerization and thus improve the rate of the desired living reaction, camphor sulfonic acid

(CSA) can be added. Upon the addition of a small amount of CSA, the isomerized dimer, **6,** is formed and autopolymerization is avoided, therefore, polymers of high molecular weight and narrow polydispersity can be synthesized. Without CSA, radical initiators **7** and **8** form and polymerization can begin, as illustrated in Scheme IV.

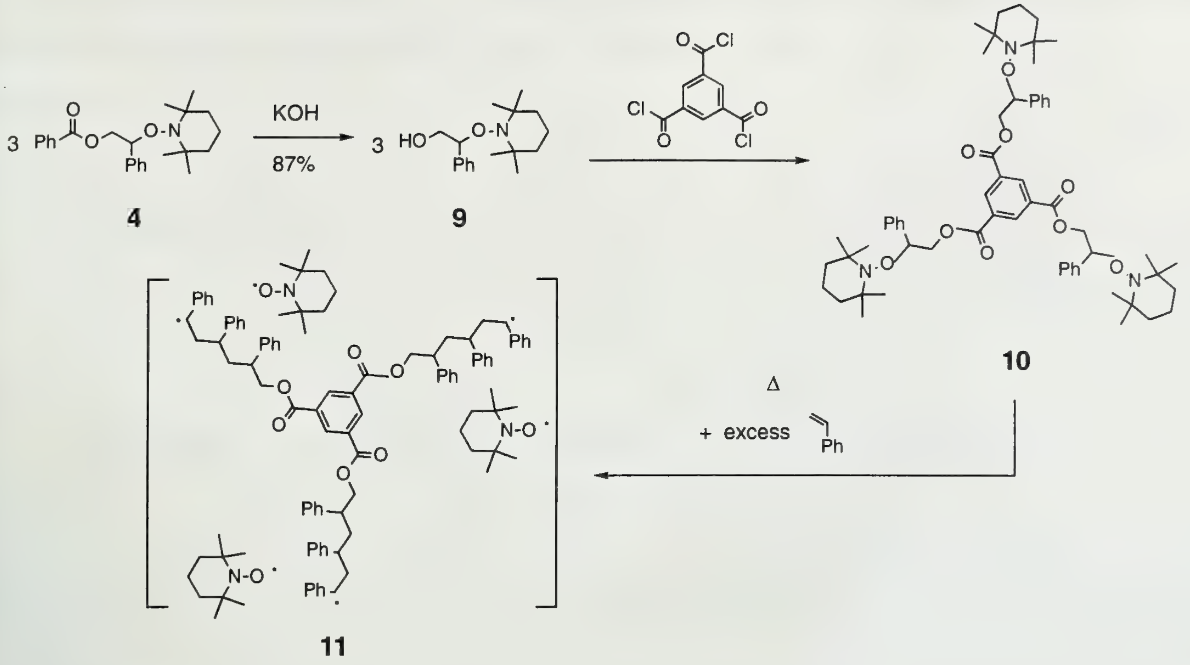
Scheme IV



VARIATIONS OF LIVING FREE RADICAL POLYMERIZATIONS

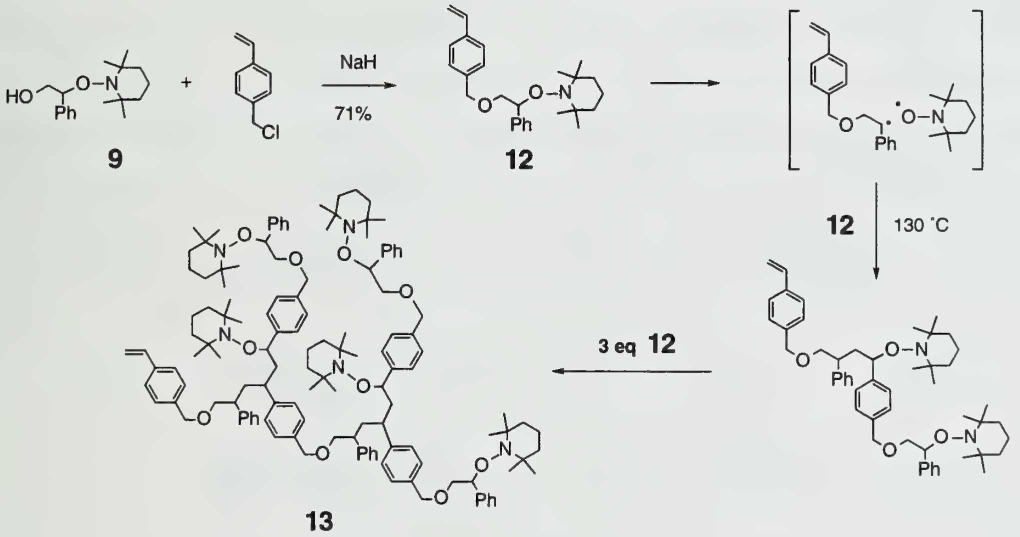
Below 100 °C, a mixture of BPO, styrene, and TEMPO yields a "modified" initiator, **4** in Scheme II, which is extremely stable.[15,23] This TEMPO-based initiator can be isolated, purified, characterized and functionalized at room temperature. For example, the ester functionality on **4** can be hydrolyzed in 87% yield to the resulting alcohol, **9** as in Scheme V. The alcohol can then be transformed into other functional groups.[24] A tridirectional "core" initiator, **10**, has been synthesized using three equivalents of **9** and 1,3,5-benzenetricarbonyl chloride.[25] Upon the addition of monomers and heat, a star polymer, **11**, can be synthesized by the living polymerization method. This structure shows no evidence of crosslinking and has a narrow polydispersity.[24] Therefore, the synthesis of modified initiators that have the ability to polymerize in a living manner can yield unique structures such as star polymers,[25] graft copolymers,[26] hyperbranched polymers,[27] and polymers of other interesting architectures.

Scheme V



A living hyperbranched polymer, **13**, can be synthesized by the self-polymerization of **12** at 130 °C as shown in Scheme VI.[27] Compound **12** serves as the initiator as well as the monomer, and is synthesized by reacting **9** with *p*-chloromethylstyrene.[25] Extended branching is due to the formation of new radical sites each time the monomer couples to the growing cluster. Living polymerizations allow hyperbranched molecules and potentially other macromolecular structures to be easily synthesized.

Scheme VI

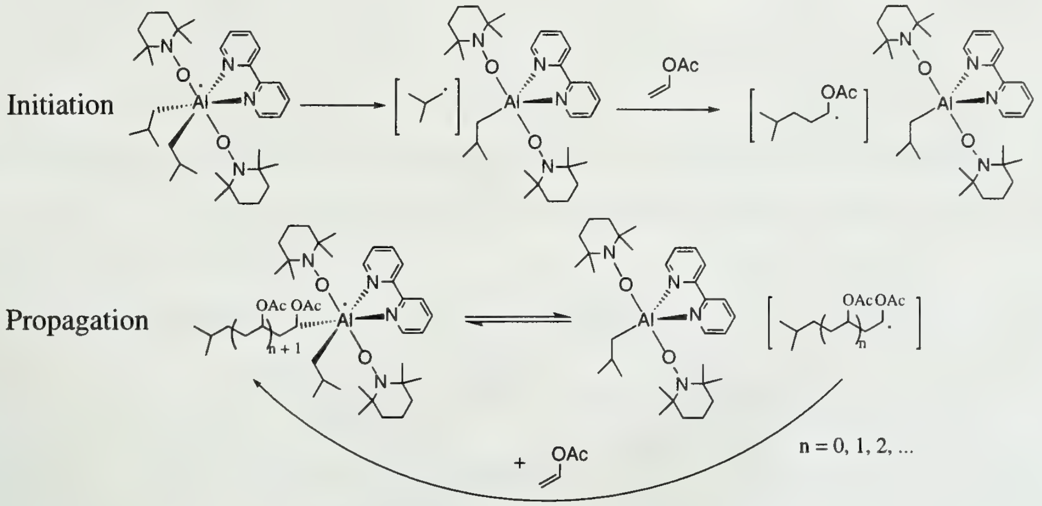


METALLIC LIVING FREE RADICAL POLYMERIZATIONS

Free radical polymerizations in which transition metals act as reversible terminators[28-31] or as catalysts in atom transfer additions[5,7,32-35] have also been determined to be living. In one such system, an organoaluminum complex with an electron donating ligand, nitroxyl radicals, and alkyl groups has the

ability to release and accept a radical.[29,30] This radical transfer allows the aluminum complex to reversibly terminate a growing vinyl acetate polymer chain, as illustrated in Scheme VII.

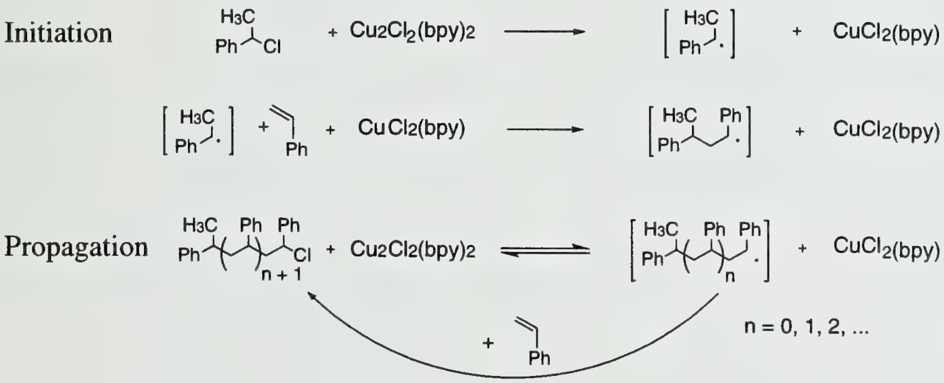
Scheme VII



In a manner similar to the aluminum complex, organometallic cobalt tetramesitylporphyrinato neopentyl complexes can initiate and control polymerizations.[31] In this system, radicals are produced upon cobalt-carbon bond homolysis. The carbon-centered radical then adds to acrylate monomers and is protected by reversible termination by the cobalt radical. Both aluminum and cobalt complexes help to stabilize radical chain addition and, therefore, allow the formation of polymers with high molecular weights and narrow polydispersities by a living mechanism.

Alkyl halides, in the presence of transition metal catalysts, can polymerize *via* the atom transfer radical addition reaction.[5,7,32-35] Atom transfer is a mechanism where dormant alkyl intermediates with carbon-chlorine terminal bonds reversibly convert into growing radical species by a redox reaction with a transition metal, as illustrated in Scheme VIII. This transfer process has been used to successfully control radical polymerizations. The polymerization of styrene, initiated by 1-phenylethyl chloride in the presence of a Cu(I)Cl complex with 2,2'-bipyridine as the catalyst, yields well-defined high molecular weight polymers with narrow polydispersity (PDI = 1.3), characteristic of a living system.

Scheme VIII



COMPARISON OF POLYMERIZATION METHODS

Monodisperse polymer chains offer a wide range of opportunities for synthesizing structures with well-defined molecular weights, architectures, and functionality and may lead to materials with unique physical properties.[36,37] Although living free radical polymerizations typically produce polymer chains that have a polydisperisty distribution larger than the living anionic, cationic, and ROMP systems, the polydisperisty of the living radical system is significantly less than that of the conventional radical process, as shown in Table II. Therefore, the living free radical polymerization method shows promise for the creation of novel polymeric materials.

Table II. Styrene Polydispersity Indices for Various Methods

Styrene Polymerization	PDI
Living-Ideal[1]	1.00
Living-Anionic/Cationic[2]	1.02 - 1.19
Living-Radical[4]	1.18 - 1.29
Conventional-Radical[4]	2.00- 4.00

CONCLUSION

Living free radical polymerization has been shown to be viable as long as the propagating free radical is protected from disproportionation or combination.[9,17,28] Therefore, to control monomer addition, the terminating radical must be capped with a labile group. Successful radical living polymerizations use nitroxide-based radicals,[17] organometallic radical stabilizers,[29,31] or transition metals with alkyl halides[33,34] as reversibly terminating agents. Each of these living free radical polymerization systems can be used to reproducibly create macromolecules with structurally well-defined and synthetically unique architectures.

REFERENCES

(1) Odian, G. *Principles of Polymerization;* 3 ed.; Wiley Interscience: New York, 1991.

(2) Keii, T.; Doi, Y.; Soga, K. In *Encyclopedia of Polymer Science and Engineering*; 2 ed.; J. I. Kroschwitz, Ed.; John Wiley & Sons Inc.: New York, 1989; Vol. Supplement; pp 380-445.

(3) Curran, D. P. In *Comprehensive Organic Synthesis*; B. M. Trost and I. Fleming, Ed.; Pergamon: Oxford, 1991; Vol. 4; pp 715.

(4) Georges, M. K.; Veregin, R. P. N.; Kazmaier, P. M.; Hamer, G. K. *Trends Polym. Sci.* **1994**, *2*, 66-71.

(5) Wang, J. S.; Matyjaszewski, K. *J. Am. Chem. Soc.* **1995**, *117*, 5614-5615.

(6) Webster, O. W. *Science* **1991**, *251*, 887-893.

(7) Matyjaszewski, K. *J. Phys. Org. Chem.* **1995**, *8*, 197-207.

(8) Ivan, B. *Macromol. Symp.* **1994**, *88*, 201-215.

(9) Otsu, T.; Yoshida, M. *Makromol. Chem., Rapid Commun.* **1982**, *3*, 127-132.

(10) Otsu, T.; Yoshida, M.; Tazaki, T. *Makromol. Chem., Rapid Commun.* **1982**, *3*, 133-140.

(11) Turner, S. R.; Blevins, R. W. *Polym. Prepr., Am. Chem. Soc., Div. Polym. Chem.* **1988**, *29*, 6-7.

(12) Moad, G.; Rizzardo, E.; Solomon, D. H. *Macromolecules* **1982**, *15*, 909-914.

(13) Bowry, V. W.; Ingold, K. U. *J. Am. Chem. Soc.* **1992**, *114*, 4992-4996.

(14) Beckwith, A. L. J.; Bowry, V. W.; O'Leary, M.; Moad, G.; Rizzardo, E.; Solomon, D. H. *J. Chem. Soc., Chem. Commun.* **1986**, 1003-1004.

(15) Moad, G.; Rizzardo, E.; Solomon, D. H. *J. Makromol. Sci.* **1982**, *A17(1)*, 51-59.

(16) Kazmaier, P. M.; Moffat, K. A.; Georges, M. K.; Veregin, R. P. N.; Hamer, G. K. *Macromolecules* **1995**, *28*, 1841-1846.

(17) Georges, M. K.; Veregin, R. P. N.; Kazmaier, P. M.; Hamer, G. K. *Macromolecules* **1993**, *26*, 2987-2988.

(18) Georges, M. K.; Veregin, R. P. N.; Kazmaier, P. M.; Hamer, G. K.; Saban, M. *Macromolecules* **1994**, *27*, 7228-7229.

(19) Veregin, R. P. N.; Georges, M. K.; Kazmaier, P. M.; Hamer, G. K. *Macromolecules* **1993**, *26*, 5316-5320.

(20) Veregin, R. P. N.; Georges, M. K.; Hamer, G. K.; Kazmaier, P. M. *Macromolecules* **1995**, *28*, 4391-4398.

(21) Mayo, F. R. *J. Am. Chem. Soc.* **1968**, *90*, 1289-1295.

(22) Georges, M. K.; Kee, A. K.; Veregin, R. P. N.; Hamer, G. K.; Kazmaier, P. M. *J. Phys. Org. Chem.* **1995**, *8*, 301-305.

(23) Hawker, C. J. *J. Am. Chem. Soc.* **1994**, *116*, 11185-11186.

(24) Hawker, C. J.; Hedrick, J. L. *Macromolecules* **1995**, *28*, 2993-2995.

(25) Hawker, C. J. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1456-1459.

(26) Georges, M. K.; Veregin, R. P. N.; Hamer, G. K.; Kazmaier, P. M. *Macromol. Symp.* **1994**, *88*, 89-103.

(27) Hawker, C. J.; Frechet, J. M. J.; Grubbs, R. B.; Dao, J. *J. Am. Chem. Soc.* **1995**, *117*, 10763-10764.

(28) Greszta, D.; Mardare, D.; Matyjaszewski, K. *Macromolecules* **1994**, *27*, 638-644.

(29) Mardare, D.; Matyjaszewski, K. *Macromolecules* **1994**, *27*, 645-649.

(30) Matyjaszewski, K. *J. Macromol. Sci., Pure Appl. Chem.* **1994**, *A31(8)*, 989-1000.

(31) Wayland, B. B.; Poszmik, G.; Mukerjee, S. L. *J. Am. Chem. Soc.* **1994**, *116*, 7943-7944.

(32) Wang, J. S.; Matyjaszewski, K. *Macromolecules* **1995**, *28*, 7572-7573.

(33) Percec, V.; Barboiu, B. *Macromolecules* **1995**, *28*, 7970-7972.

(34) Wang, J. S.; Matyjaszewski, K. *Macromolecules* **1995**, *28*, 7901-7910.

(35) Matyjaszewski, K.; Gaynor, S.; Greszta, D.; Mardare, D.; Shigemoto, T. *J. Phys. Org. Chem.* **1995**, *8*, 306-315.

(36) Fréchet, J. M. J. *Science* **1994**, *263*, 1710-1715.

(37) Fréchet, J. M. J.; Henmi, M.; Gitsov, I.; Aoshima, S.; Leduc, M. R.; Grubbs, R. B. *Science* **1995**, *269*, 1080-1083.

POLYMER SUPPORTED COMBINATORIAL SYNTHESIS OF SMALL ORGANIC MOLECULES

Reported by Samir Serrano December 4, 1995

INTRODUCTION

Currently there are four ways of discovering biologically active compounds: screening of chemical libraries and natural products mixtures, serendipity, rational design, and computer-assisted drug design.[1] However, with the development of high throughput screening techniques, which can screen thousands of compounds per day, more compounds are needed for screening.[2] Combinatorial chemistry has recently emerged as a way to analyze structure-activity relationships (SAR) by screening a large array of compounds synthesized in a predictably random manner, without the inconveniences of molecular isolation and purification.[3] Combinatorial synthesis is defined as the parallel synthesis of a vast array of compounds by assembling components of differing functionalities, where the number of compounds prepared is greater than the number of chemical steps required.[4] This review will focus on recent efforts to apply organic synthesis and methodologies to the construction of combinatorial libraries which produce small organic molecules and will give some preliminary bioactivity information.

BACKGROUND

Merrifield's solid phase method for the synthesis of peptides and oligonucleotides, is the basis upon which combinatorial chemistry is built.[5,6] Recently there have been reports of similar methods for the synthesis of oligosaccharides.[7] Even though all three types of compounds show high activity *in vitro*, they are often not pursued as drug candidates due to their low bioavailability, poor oral efficacy, and limited *in vivo* stability.[1,8,9] Chemists and biochemists have therefore tried to synthesize molecules that are resistant to enzymatic degradations, peptoids for example.[9,10] However, it is known that high molecular weights limit the oral bioavailability of a compound.[11] Thus, molecules with molecular weights less than 750 g/mol, referred to as small organic molecules, are being investigated.[2,12,13] Combinatorial synthesis allows the synthesis of thousands of compounds expediently, conveniently, and efficiently using parallel synthesis. Parallel synthesis is the simultaneous formation of several hundreds of compounds with the same general structure, but differing functional groups. All of these compounds are then screened or tested for a specific biological activity with the hope that a potential drug candidate can be uncovered.

There are several requirements which must be met in order to successfully construct a combinatorial library. Polymeric supports have traditionally been used for the synthesis of combinatorial libraries, since they allow facile purification of products, and reactions can be driven to completion by using

Copyright © 1995 by Samir Serrano

excess reagent.[13] This allows for the immediate use of recently synthesized compounds without the need of further purification. The compounds in combinatorial libraries should be diverse, fairly complex, easily accessible, with a wide range of physiochemical properties and functionality, and a high density of functional groups.[4,12] The coupling reactions should also be orthogonal to the removal of the compound from the polymeric support.[12] Many of the compounds presented here are derived from traditional pharmacophores (e.g. benzodiazepines, β-lactams, imidazoles, phenethylamines, hydantoins) due to their proven efficacy as therapeutic agents.[12,13] In contrast, some of the other compounds presented here were constructed to demonstrate the wide applicability of combinatorial libraries using a polymer-support.

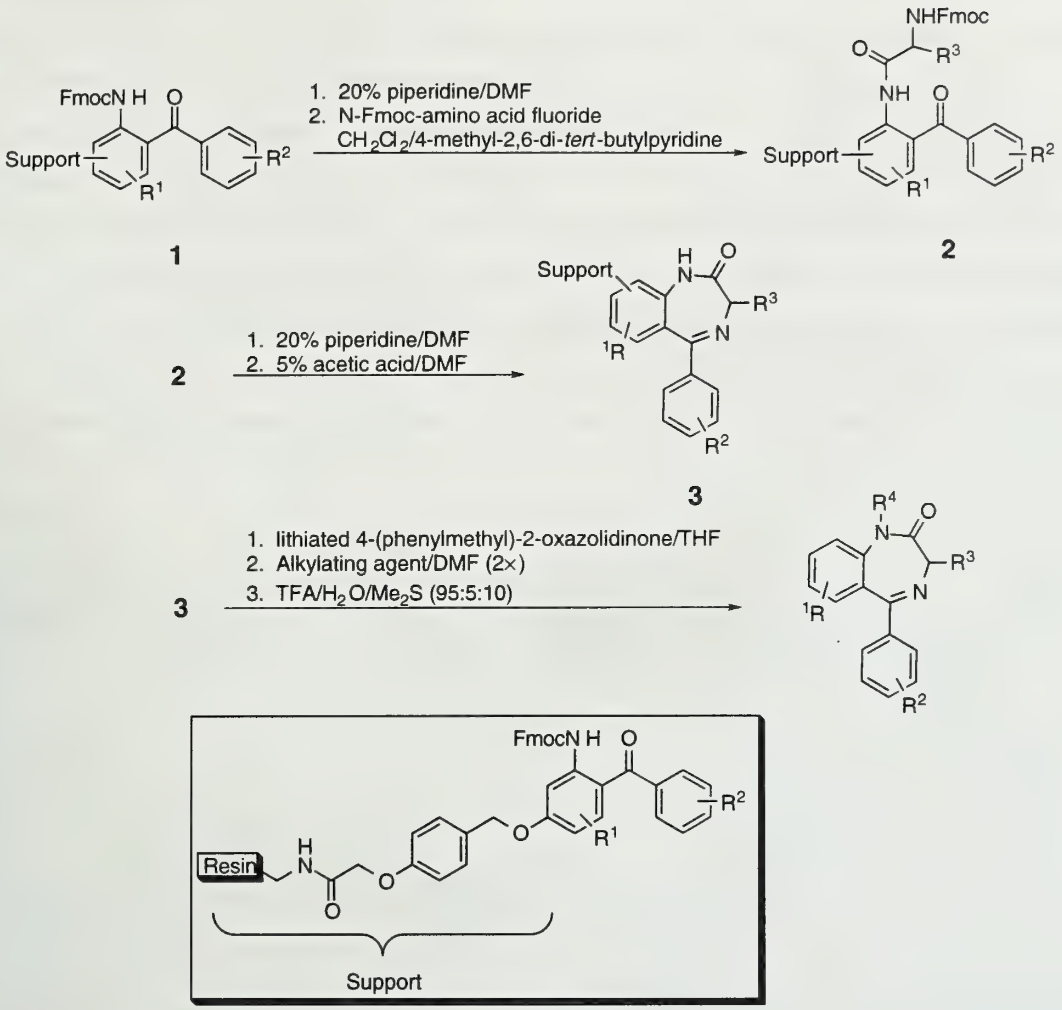
BENZODIAZEPINES[14]

One of the best known benzodiazepines is Valium, an anxiolytic and skeletal muscle relaxant.[15] These compounds have other biological activity including anticonvulsant, antihypnotic, cholecystokinin (CCK) receptor A and receptor B antagonists, opioid receptor ligands, platelet-activating factor antagonists, human immunodeficiency virus trans-activator Tat antagonists, GP-IIbIIIa inhibitors, reverse transcriptase inhibitors, and ras farnesyltransferase inhibitors.[14b,c] Benzodiazepines have been synthesized by Ellman and coworkers using hydroxy-functionalized 2-aminobenzophenone derivatives (Scheme I) or 2-aminoaryl ketones.[14c] The aminobenzophenone method was selected because of the number of protected natural and unnatural amino acids, and alkylating agents which are commercially available. Both activated and unactivated alkylating reagents were used for this synthesis (e.g. methyl iodide, benzyl bromide, iodoacetonitrile, alkenes, alkynes, ethyl iodide). This methodology yielded 192 distinct compounds with no racemization (≤2%) of the amino acids with a low yield of 67% and an average yield of 86%. The authors estimate that >10,000 different benzodiazepines could be synthesized in about 4 weeks.[14b] A cholecystokinin A receptor (CCK A) binding affinity assay of the synthesized compounds revealed that the incorporation of D- or L-tryptophan, as the amino acid, increased the binding of the benzodiazepine.

Ellman and co-workers also synthesized a benzodiazepine library containing 2-aminoaryl ketones. The ketone functionality introduces an essential determinant of activity or specificity for these compounds.[14c] However, there are relatively few commercially available 2-aminoaryl ketones. The synthesis circumvents this problem by preparing the ketones on the support through a Stille coupling reaction, then carrying out the cyclization as in Scheme I. Yields obtained for this reaction were between 52% and 82%.

DeWitt and co-workers have also synthesized benzodiazepines, albeit their approach was different.[14d] They used an imine to couple the aminoaryl ketone to a resin-bound amino acid. Yields for the 40 compounds synthesized ranged from 9% to 63% with purities >90%.

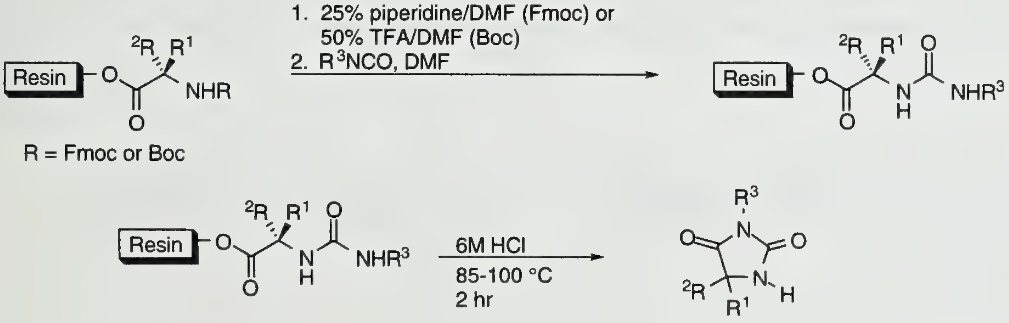
SCHEME I


FmocN H O
Support
R1
R2
1
1. 20% piperidine/DMF
2. N-Fmoc-amino acid fluoride
CH2Cl2/4-methyl-2,6-di-tert-butylpyridine
NHFmoc
O
R3
NH O
2
1. 20% piperidine/DMF
2. 5% acetic acid/DMF
H O
N
3
1. lithiated 4-(phenylmethyl)-2-oxazolidinone/THF
2. Alkylating agent/DMF (2×)
3. TFA/H2O/Me2S (95:5:10)
R4
Resin
H
N
O
Support

HYDANTOINS[14d]

DeWitt and co-workers have also reported the synthesis of hydantoins. Hydantoins, such as Dilantin, are reported to have anticonvulsant and antiepileptic effects.[15] These compounds were synthesized on a polymeric support (Scheme II) with total yields between 4% and 81% for 39 compounds. An interesting aspect of this synthesis is the fact that the cyclization reaction separates the final product from the intermediates that did not cyclize, since acyclic compounds are not released from the resin.

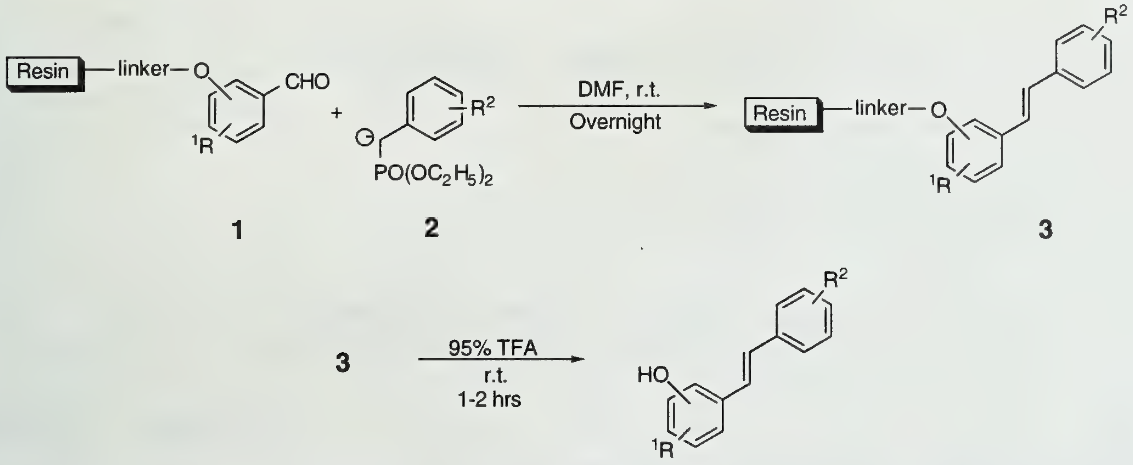
SCHEME II



HYDROXYSTILBENES[17]

Hydroxystilbenes are non-steroidal estrogen agonists which are very important in mediating cellular responses. Estrogens are used in hormone replacement therapy for postmenopausal women. Scanlan and co-workers were able to synthesize a variety of hydroxystilbenes on a solid support using combinatorial library techniques together with Horner-Emmons olefination chemistry (Scheme III). The 23-compound library gave yields between 7% and 85% (≥ 5 mg) and good purity as determined by TLC, NMR and mass spectrometry. The screening procedure yielded some compounds that can elicit the transcription of an estrogen responsive gene. The investigators were also able to draw some preliminary SAR conclusions. A hydroxyl substituent para to the stilbene olefin is a requirement for biological activity. An unsubstituted or fluorine substituted distal aromatic ring shows the highest affinity when coupled with the para hydroxy substituent.

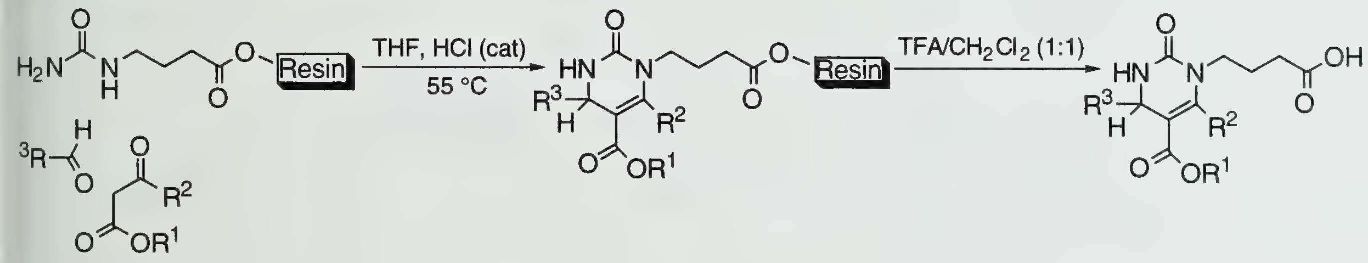
SCHEME III



SOLID PHASE APPLICATION OF THE BIGINELLI DIHYDROPYRIMIDINE SYNTHESIS[18]

The Biginelli synthesis is a multi component condensation (β-ketoester + aldehyde + urea) that yields products with a high density of functionality in one step. This condensation also yields heterocycles which have shown pharmacological activity (antimicrobial, antiviral, antitumor, antiinflammatory, and cardiovascular activities). The solid phase version proceeds by attaching the urea to the resin and then allowing it to condense with the β-ketoester and the aldehyde (Scheme IV). Yields ranged from 67% to 98% with purities greater than 95%. These yields are superior by 10-20% to the classical solution synthesis. Moreover the practitioner was spared the purification of the compounds by crystallization or chromatographic means.

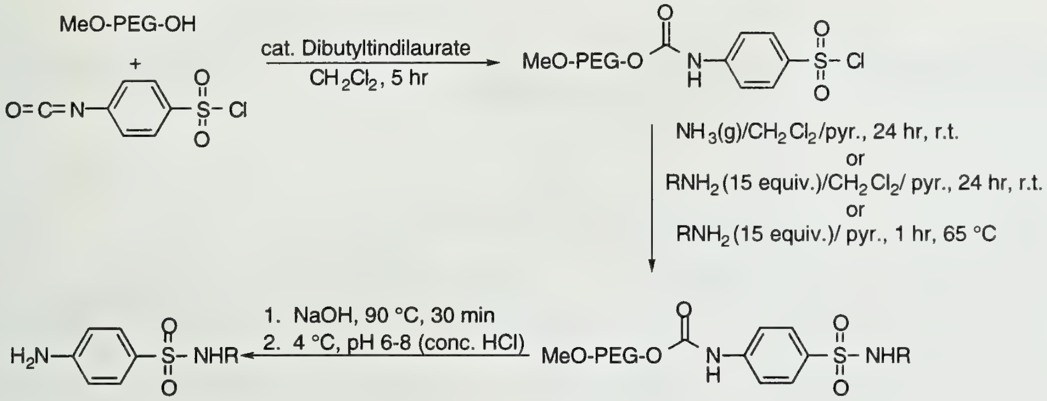
SCHEME IV



ARYLSULFONAMIDES SYNTHESIZED ON SOLUBLE POLYMER SUPPORT[19]

Janda and co-workers have used Bayer and Mutter's soluble polymer support[20], monomethyl polyethylene glycol (MeO-PEG-OH), to synthesize a small library of sulfonamides (Scheme V). These compounds have potential antiarrhythmic and antitumor activities, and endothelin antagonist properties.[21] The soluble support is noteworthy, since it allows the user to carry out the reaction in a diverse range of solvents (H_2O, DMF, CH_2Cl_2, MeOH,) as a liquid phase reaction. An advantage to this method is that the reaction is homogeneous and not subject to nonlinear kinetic behavior as solid resins usually are.[19] Also, the reactions can be easily followed by spectroscopic means such as UV, NMR, and IR among others. When diethyl ether is added to the solution, the soluble polymer precipitates, making it easy to wash and purify the products. The products afforded were analytically pure with yields between 95% and 97%.

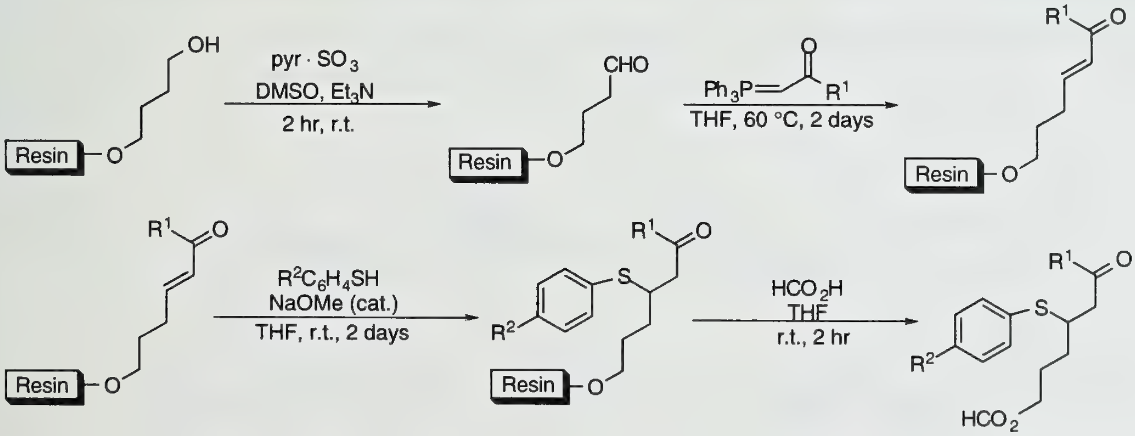
SCHEME V


MeO-PEG-OH
+
O=C=N
O
S- Cl
O
cat. Dibutyltindilaurate
CH_2Cl_2, 5 hr
MeO-PEG-O
N
H
NH_3(g)/CH_2Cl_2/pyr., 24 hr, r.t.
or
RNH_2 (15 equiv.)/CH_2Cl_2/ pyr., 24 hr, r.t.
or
RNH_2 (15 equiv.)/ pyr., 1 hr, 65 °C
1. NaOH, 90 °C, 30 min
2. 4 °C, pH 6-8 (conc. HCl)
H_2N
S- NHR

β-MERCAPTO KETONES[22]

Kurth and co-workers have prepared β-mercapto ketones to see if these compounds are adaptable to combinatorial synthesis. In the synthesis of their modest library (9 compounds), they were able to use pyridine/sulfur trioxide oxidation, Horner-Emmons condensation, and Michael addition reactions on the resin bound substrate to afford the final product (Scheme VI). While their final yields were low (7-27%), the final product was relatively pure. Each transformation in this scheme was followed by KBr pellet FT-IR analysis.

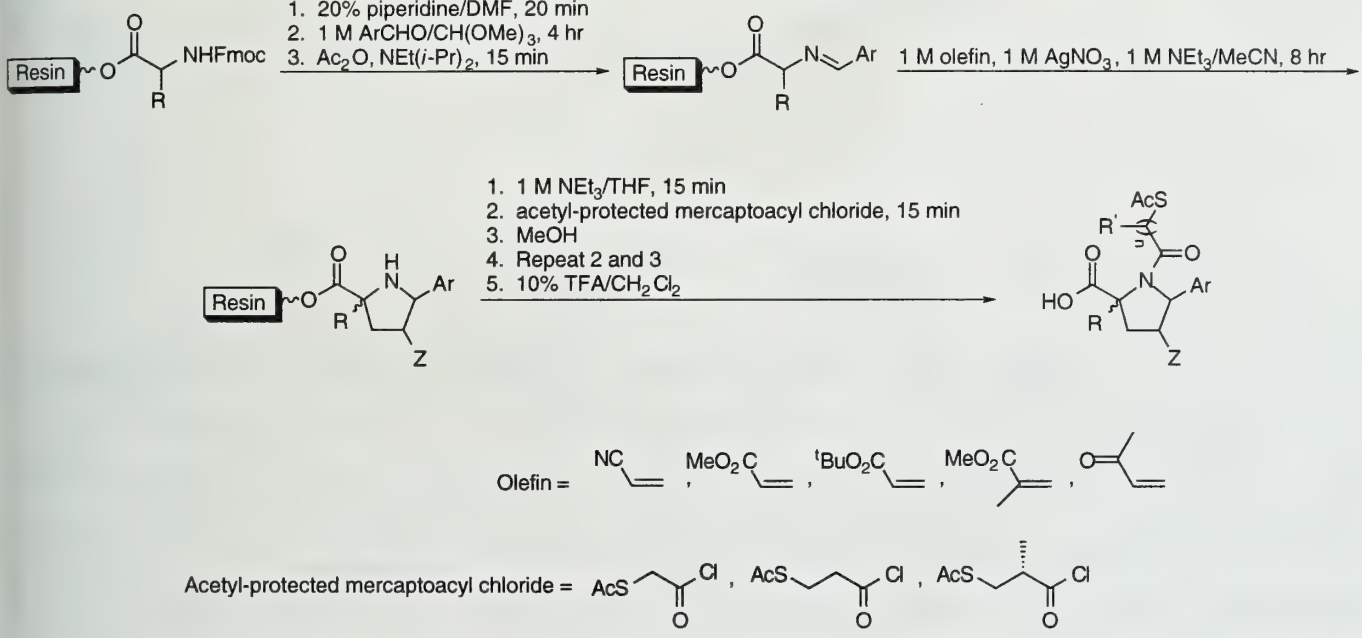
SCHEME VI



HIGHLY FUNCTIONALIZED PYRROLIDINES[23]

Gallop and co-workers at Affymax have made a library of highly functionalized pyrrolidines and, from these, they have identified a potent angiotensin converting enzyme (ACE) inhibitor. The crucial assembly was obtained by a 1,3-dipolar cycloaddition to the resin-bound azomethine ylide (Scheme VII). For six model members of this library, which consisted of 480 compounds, their yields ranged from 50-80% with diastereoselectivities ranging from 2.5:1 to greater than 10:1. An SAR study of the compounds revealed that use of glycine as the amino acid, benzaldehyde as the imine forming compound, methyl acrylate as the 1,3-dipolar cycloaddition olefin, and 2(*S*)-3-acetyl mercaptoisobutyric chloride as the acylating agent produced the compound with the highest inhibitory activity (IC_{50} = 2 nM). This compound is 3 times more active than Captopril, currently used as an ACE inhibitor.

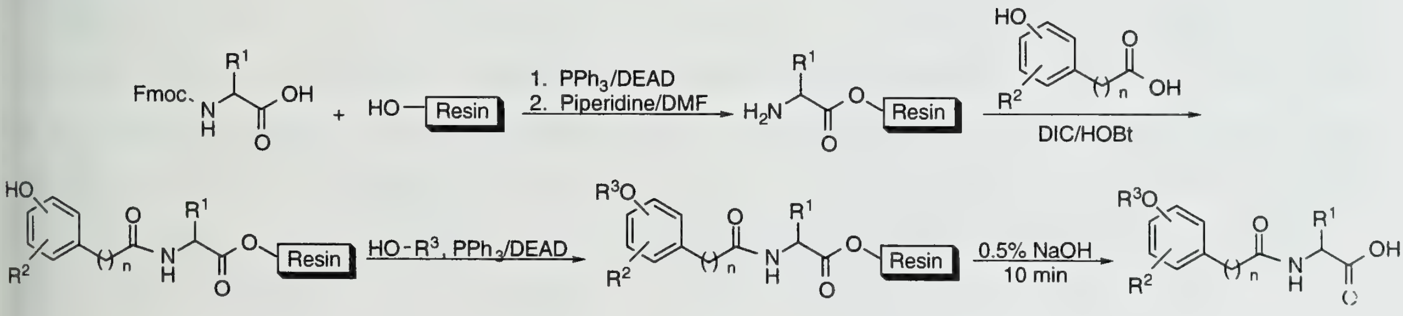
SCHEME VII


Resin
NHFmoc
R
1. 20% piperidine/DMF, 20 min
2. 1 M ArCHO/CH(OMe)$_3$, 4 hr
3. Ac$_2$O, NEt(i-Pr)$_2$, 15 min
N
Ar
1 M olefin, 1 M AgNO$_3$, 1 M NEt$_3$/MeCN, 8 hr
Z
1. 1 M NEt$_3$/THF, 15 min
2. acetyl-protected mercaptoacyl chloride, 15 min
3. MeOH
4. Repeat 2 and 3
5. 10% TFA/CH$_2$Cl$_2$
AcS
R'
HO
Olefin =
NC
MeO$_2$C
^{t}BuO$_2$C
Acetyl-protected mercaptoacyl chloride =
Cl

MITSUNOBU ETHER FORMATION[24]

Krchnák and co-workers synthesized a model library of 4,200 different alkyl aryl ethers, each attached to a resin, using the Mitsunobu ether formation reaction (Scheme VIII).[24] This was accomplished using all natural amino acids, 10 aromatic hydroxy acids, and 21 alcohols. A smaller scale optimization reaction was performed before the synthesis of this library with 60% to 95% yields.

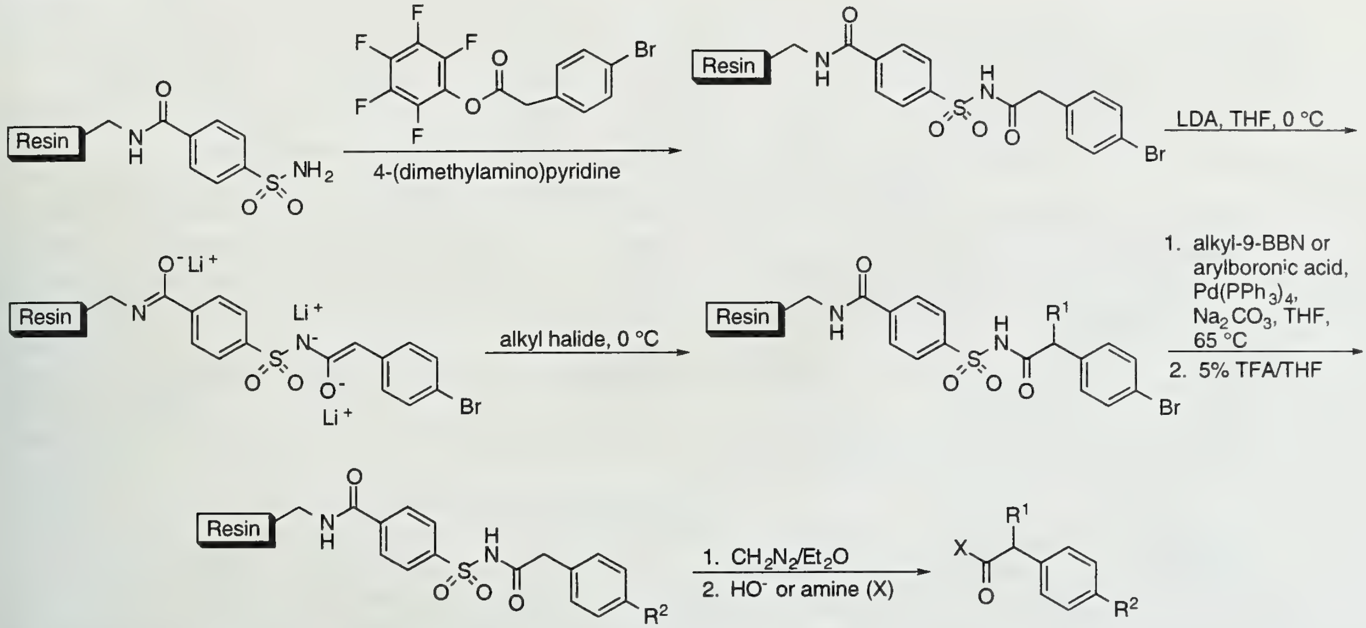
SCHEME VIII



ARYL ACETIC ACID DERIVATIVES[25]

Ellman also reported the synthesis of substituted arylacetic acid derivatives, a class of cyclooxygenase inhibitors. First enolate chemistry was used to alkylate the α-carbon of an amide and then the aryl group was alkylated by a Suzuki coupling (Scheme IX).[25] Twelve compounds were made, with yields between 87% and 100%.

SCHEME IX



OTHER ORGANIC REACTIONS IN COMBINATORIAL SYNTHESIS

There have also been recent reports of the combinatorial synthesis of C_2 symmetric inhibitors of HIV protease using a solid support.[26] Steele and co-workers have reported the construction of a 1,000 component library of piperazinediones with the lowest yields being about 20%.[27] Terrett and co-workers were involved in the synthesis of a 30,752 compound library of possible endothelin antagonists.[28] Dankwardt and co-workers have recently reported the solid phase synthesis of aryl and benzylpiperazines and their applications in combinatorial synthesis.[29]

CONCLUSION

Many examples exist in the literature of organic reactions and methodologies that have been used in conjunction with a polymeric support to build combinatorial libraries of small organic compounds. The use of a polymeric support has simplified and shortened purification, allowing for the rapid synthesis and screening of compounds to yield potential drug candidates. The automation of this technique can be foreseen, as well as the addition of screening assays as part of the automation. However, caution is given to not abandon the search for new reactions and methodologies in organic chemistry since these constitute the base on which combinatorial synthesis will thrive.[1] The application of more organic reaction methodologies to polymer-supported synthesis and optimization of conditions presents an exciting and extremely useful topic of research in industrial and academic settings.[2]

References

1. Mitscher, L.A. *Chemtracts-Organic Chemistry* **1995**, *8*, 19.
2. Czarnik, A.W. *Chemtracts-Organic Chemistry* **1995**, *8*, 13.
3. Koppel, G.; Dodds, C.; Houchins, B.; Hunden, D.; Johnson, D.; Owens, R.; Chaney, M.; Usdin, T.; Hoffman, B.; Brownstein, M. *Chemistry & Biology* **1995**, *2*, 483.
4. Pirrung, M.C. *Chemtracts-Organic Chemistry* **1995**, *8*, 5.
5. Merrifield, R.B. *J. Am. Chem. Soc.* **1963**, *85*, 2149.
6. Letsinger, R.L.; Mahadevan, V. *J. Am. Chem. Soc.* **1965**, *87*, 3526.
7. a) Wang, Y.; Zhang, H.; Voelter, W. *Chemistry Letters* **1995**, 273. b) Douglas, S.P.; Whitfield, D.M.; Krepinsky, J.J. *J. Am. Chem. Soc.* **1995**, *117*, 2116. c) Yan, L.; Taylor, C.M.; Goodnow. R., Jr.; Kahne, D. *J. Am. Chem. Soc.* **1994**, *116*, 6953. d) Danishefsky, S.J.; McClure, K.F.; Randolph, J.T.; Ruggeri, R.B. *Science* **1993**, *260*, 1307. e) Verduyn, R.; van der Klein, P.A.M.; Douwes, M.; van der Marel, G.A.; van Boom, J.H. *Recl. Trav. Chim. Pays-Bas* **1993**, *112*, 464.
8. Zuckermann, R.N. *Current Opinion in Structural Biology* **1993**, *3*, 580.
9. Simon, R.J.; Kania, R.S.; Zuckerman, R.N.; Huebner, V.D.; Jewell, D.A.; Banville, S.; Ng, S.; Wang, L.; Rosenberg, S.; Marlowe, C.K.; Spellmeyer, D.C.; Tan, R.; Frankel, A.D.; Santi, D.V.; Cohen, F.E.; Bartlett, P.A. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 9367.
10. Zuckermann, R.N.; Kerr, J.M.; Kent, S.B.H.; Moos, W.H. *J. Am. Chem. Soc.* **1992**, *114*, 10646.
11. Martin, E.J.; Blaney, J.M.; Siani, M.A.; Spellmeyer, D.C.; Wong, A.; Moos, W.H. *J. Med. Chem.* **1995**, *38*, 1431.
12. Gordon, E.M.; Barrett, R.W.; Dower, W.J.; Fodor, S.P.A.; Gallop, M.A. *J. Med. Chem.* **1994**, *37*, 1385.
13. Ellman, J.A. *Chemtracts-Organic Chemistry* **1995**, *8*, 1.
14. a) Bunin, B.A.; Ellman, J.A. *J. Am. Chem. Soc.* **1992**, *114*, 10997. b) Ellman, J.A.; Bunin, B.A.; Plunkett, M.J. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 4708. c) Ellman, J.A.; Plunkett, M.J. *J. Am. Chem. Soc.* **1995**, *117*, 3306. d) DeWitt, S.H.; Kiely, J.S.; Stankovic, C.J.; Schroeder, M.C.; Cody, D.M.R.; Pavia, M.R. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 6909.
15. Diazepam (compound # 2967) in *Merck Index*, 10th ed.; Windholz, M., Ed.; Merck & Co: Rahway, New Jersey, 1983; p 433.
16. Phenytoin (Dilantin, compound # 7204) in *Merck Index*, 10th ed.; Windholz, M., Ed.; Merck & Co: Rahway, New Jersey, 1983; p 1054.
17. Williard, R.; Jammalamadaka, V.; Zaua, D.; Benz, C.C.; Hunt, C.A.; Kushner, P.J.; Scanlan, T.S. *Chemistry & Biology* **1995**, *2*, 45.
18. Wipf, P.; Cunningham, A. *Tetrahedron Lett.* **1995**, *36*, 7819.
19. Han, H.; Wolfe, M.M.; Brenner, S.; Janda, K.D. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 6419.
20. Bayer, E.; Mutter, M. *Nature(London)* **1972**, *237*, 512.
21. a) Ellingboe, J.W.; Spinelli, W.; Winkley, M.W.; Nguyen, T.T.; Parsons, R.W.; Moubarak, I.F.; Kitzen, J.M.; Von Engen, D.; Bagli, J.F. *J. Med. Chem.* **1992**, *35*, 705. b) Yoshino, H.; Ueda, N.; Niijima, J.; Sugumi, H.; Kotake, Y.; Koyanagi, N.; Yoshimatsu, K.; Asada, M.; Watanabe, T.; Naasu, T.; Tsukahara, K.; Iijima, A.; Kitoh, K. *J. Med. Chem.* **1992**, *35*, 2497. c) Stein, P.D.; Hunt, J.T.; Floyd, D.M.; Moreland, S.; Dickinson, K.E.J.; Mitchell, C.; Liu, E.C.-K.; Webb, M.L.; Murugesan, N.; Dickey, J.; McMullen, D.; Zhang, R.; Lee, V.G.; Serafino, R.; Delaney, C.; Schaeffer, T.R.; Kozlowski, M. *J. Med. Chem.* **1994**, *37*, 329.
22. Chen, C.; Randall, L.A.A.; Miller, R.B.; Jones, A.D.; Kurth, M.J. *J. Am. Chem. Soc.* **1994**, *116*, 2661.
23. Murphy, M.M.; Schullek, J.R.; Gordon, E.M.; Gallop, M.A. *J. Am. Chem. Soc.* **1995**, *117*, 7029.
24. Krchnák, V.; Flegelová, Z.; Weichsel, A.S.; Lebl, M. *Tetrahedron Lett.* **1995**, *36*, 6193.
25. Backes, B.J.; Ellman, J.A. *J. Am. Chem. Soc.* **1994**, *116*, 11171.
26. Wang, G.T.; Li, S.; Wideburg, N.; Krafft, G.A.; Kempf, D.J. *J. Med. Chem.* **1995**, *38*, 2995.
27. Gordon, D.W.; Steele, J. *Bioorg. Med. Chem. Let.* **1995**, *5*, 47.
28. Terrett, N.K.; Bojanic, D.; Brown, D.; Bungay, P.J.; Gardner, M.; Gordon, D.W.; Mayers, C.J.; Steele, J. *Bioor. Med. Chem. Lett.* **1995**, *5*, 917.
29. Dankwardt, S.M.; Newman, S.R.; Krstenansky, J.L. *Tetrahedron Lett.* **1995**, *36*, 4923.

RADICAL CATION CYCLOADDITIONS

Reported by Zhengguo Zhu December 7, 1995

INTRODUCTION

The Diels-Alder (D-A) reaction is one of the most important in organic synthesis. Classical D-A reactions generally involve a diene which is electron-rich and a dienophile which is electron-deficient. When the dienophile is not relatively electron-deficient, the reaction is very slow and, in some cases even affords no D-A adduct. Many versions of D-A reactions have been developed to complement the classical D-A reactions. Among these are inverse electron demand D-A, Lewis acid catalyzed D-A, the use of microwave heating, the use of water as solvent, and the use of high pressure. Never the less, not all desired D-A adducts can be constructed effectively by means of neutral D-A reactions, especially in cases where both the diene and dienophile are electron-rich or where the dienophile is ketene. Transformation of either the dienophile or the diene to its radical cation has been found to promote certain D-A reactions which proceed slowly or not at all under neutral conditions. Radical cation formation has also been used in effecting other types of cycloadditions.

RADICAL CATION DIELS-ALDER REACTION

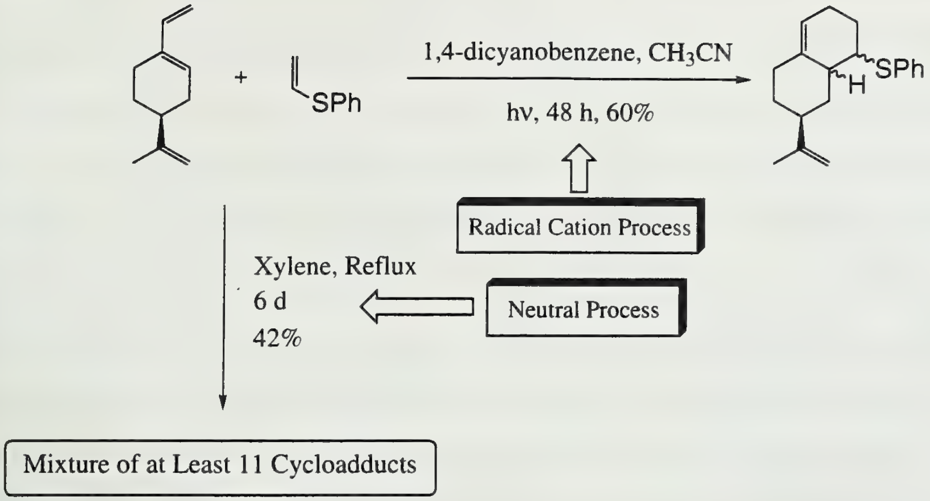
Reaction Overview

The radical cation D-A reaction is carried out via the conversion of either the diene or dienophile component to the corresponding radical cation. This conversion can be brought about through a one electron oxidation by chemical, photochemical or electrochemical methods. The enhancement of reaction rate and yield is remarkable in certain cases. In Scheme I is a typical radical cation D-A reaction is compared with the corresponding neutral D-A reaction.[1] In the preparation of D-A adduct **3** from 1-ethenyl-4-isopropenylcyclohexene, **1**, and phenylvinylsulfide, **2**, the thermal D-A reaction was found to be very inefficient because the dienophile is not sufficiently electron deficient. Heating a mixture of **1** and **2** to reflux in xylenes for 6 days yielded only 42% of a mixture of at least 11 cycloadducts of the diene and dienophiles. The D-A reaction of the same starting materials under the radical cation conditions yields **3** in 60% yield in two days.

The conditions used for radical cation D-A reactions are generally mild. For reactions where neutral D-A reactions are notoriously slow because the dienophiles used are electron rich, the radical cation D-A reaction generally works well.[2] Just as in the neutral D-A reaction, the conformation of the diene component plays an important role in radical cation process. Good results are generally obtained when the diene components are either cyclic (i.e., rigidly *s*-cis) or, if acyclic, have at least a moderate population of the *s*-cis conformations.[3] Alkyl substituents on the dienophile can increase the efficiency of the reaction.[3]

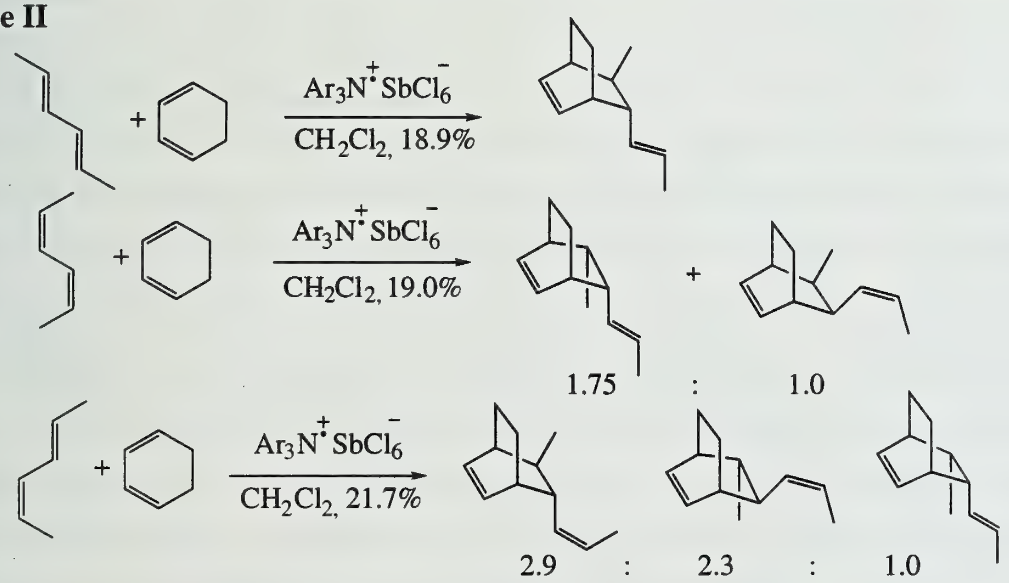
Copyright © 1995 by Zhengguo Zhu

Scheme I



Suprafacial stereospecificity, which is common to almost all radical cation D-A reactions, is demonstrated by the reactions between 1,3-cyclohexadiene and the three geometric isomers of 2,4-hexadiene (Scheme II).[2] In cases where there is an unsaturated substituent on the diene component, high *endo* selectivity (sometimes even higher than that of the neutral reaction) is observed.[2] With asymmetric dienes or dienophiles, the regioselectivity is also remarkable, in certain cases.[3,4]

Scheme II



Reaction Conditions

Triarylaminium salts ($Ar_3N^{+}\cdot X^{-}$) with *para*-substituents were found to be convenient one-electron oxidizing agents for organic molecules.[3,5] Among them, tris-(*p*-bromophenyl)aminium hexachloroantimonate is more stable with a standard redox potential of 1.28V (vs. NHE). It is commercially available and thus widely used as an initiator in the radical cation D-A. Typical conditions for chemically initiated radical cation D-A reactions are: 0-25°C, 5-10 mol% of tris-(*p*-

bromophenyl)aminium hexachloroantimonate, 5-15 min. The reaction is then quenched by the addition of excess sodium methoxide/methanol.[2] Polymers bearing radical cation functional groups were also prepared and successfully used to initiate radical cation cycloaddition.[6]

Photoinduced electron transfer (PET) is another widely used method for generating radical cations.[7,8] The principle behind this method is that some of the organic molecules (photosensitizers) are transformed to better electron acceptors once excited with light. Then they can extract one electron from other substrates. The photosensitizers used in radical cation D-A reactions are generally electron deficient aromatic species which can be excited readily with light. Among the commonly used photosensitizers are 1,4-dicyanobenzene, 2,4,6-triphenylpyrylium tetrafluoroborate (TPP), and 1,4-dicyanonaphthalene. The conditions used in the radical cation reaction shown in Scheme I are typical of this method except that, in most cases, the reaction time is much shorter. Use of polymer-bound tryphenylpyrylium salts as photosensitizers for the generation of radical cations was also reported.[7]

Radical cation D-A reactions have also been found to be initiated by electrochemical oxidation,[9] photostimulated semiconductors,[10] or γ radiolysis.[5]

Mechanistic Study

Two mechanisms have been proposed for the radical cation D-A reaction initiated by aminium salts: a radical chain mechanism and a mechanism which includes an aminium salt-alkene complex as an intermediate, as shown in Scheme III.[11] Kinetic studies on the dimerization of 1,3-cyclohexadiene catalyzed by triarylamminium salts resulted in an experimental rate law which is in good agreement with the radical cation chain mechanism with the assumption that a bimolecular chain termination step is involved.[12] The radical cation chain mechanism is also supported by the fact that D-A reactions catalyzed by aminium salts are generally very fast. However, studies of PET dimerization of various 1,3-hexadienes under different concentrations suggest that different types of dienophiles, contact ion pairs and solvent-separated ion pairs, are involved. This mechanism, which was classified as the electron-transfer sensitization mechanism, is outlined in Scheme IV.[13] In each mechanism proposed so far, it is recognized that cycloaddition between the radical cation diene and the dienophile, or between the radica cationl dienophile and the diene is the key step. This makes the reaction quite different from its neutral counterpart. The fact that the dimerization of 1,3-cyclohexadiene can be promoted under various conditions where the generation of a radical cation is conceivable strongly supports the importance of radical cations in these reactions. The generation of a radical cation diene has been further confirmed by NMR and ESR.[14] Experiments on the fluorescence quenching of the photosensitizer by cyclohexadiene derivatives also supports the involvement of radical cations.[13] Most importantly, the involvement of radical cations in the cycloaddition step can explain the phenomenal enhancements in rate, efficiency, and regio- and stereoselectivity of D-A reactions under radical cation generating conditions.

A lot of theoretical work has focused on the cycloaddition step. Due to the suprafacial selectivity of the reaction, it is believed that the reaction takes place either in a concerted manner or in a stepwise

fashion with the formation of the first bond followed by the fast formation of the second bond. Since the rate enhancement can not be explained if the concerted bond forming mechanism is assumed,[15] a "non-synchronous concerted" mechanism[4,15,16] was proposed based on simple thermodynamic and kinetic considerations and was supported by theoretical calculations. Further work suggests that the radical cycloaddition can range from non-synchronous concerted to nonsynchronous nonconcerted depending on the nature of the dienes and the dienophiles.[17] This change has led to the obversavation of antarafacial adducts in certain cases.[18]

Scheme III

Radical Cation Chain Mechanism

$$\mathrm{DP} + \mathrm{Ar_3N}^{\bullet+} \rightleftharpoons \mathrm{Ar_3N}\colon + \mathrm{DP}^{\bullet+}$$

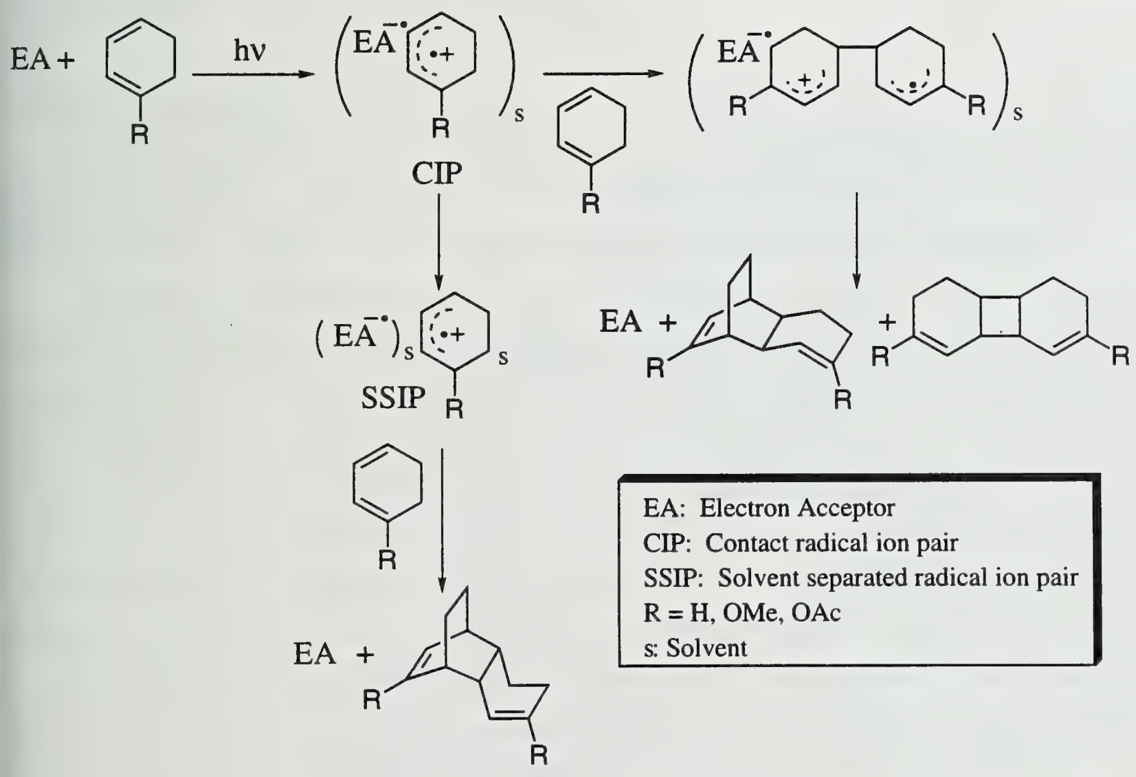
$$\left.\begin{array}{l} \mathrm{DP}^{\bullet+} + \mathrm{D} \longrightarrow \mathrm{A}^{\bullet+} \\ \mathrm{A}^{\bullet+} + \mathrm{DP} \longrightarrow \mathrm{A} + \mathrm{DP}^{\bullet+} \end{array}\right\} \text{propagation}$$

Complex Mechanism

$$\mathrm{DP} + \mathrm{Ar_3N}^{\bullet+} \rightleftharpoons \mathrm{Ar_3N}\text{--}^{+\bullet}\text{--}\mathrm{DP} \xrightarrow[\text{r.d.s.}]{\mathrm{D}} \mathrm{A} + \mathrm{Ar_3N}^{\bullet+}$$

D = diene; DP = dienophile; A = adduct

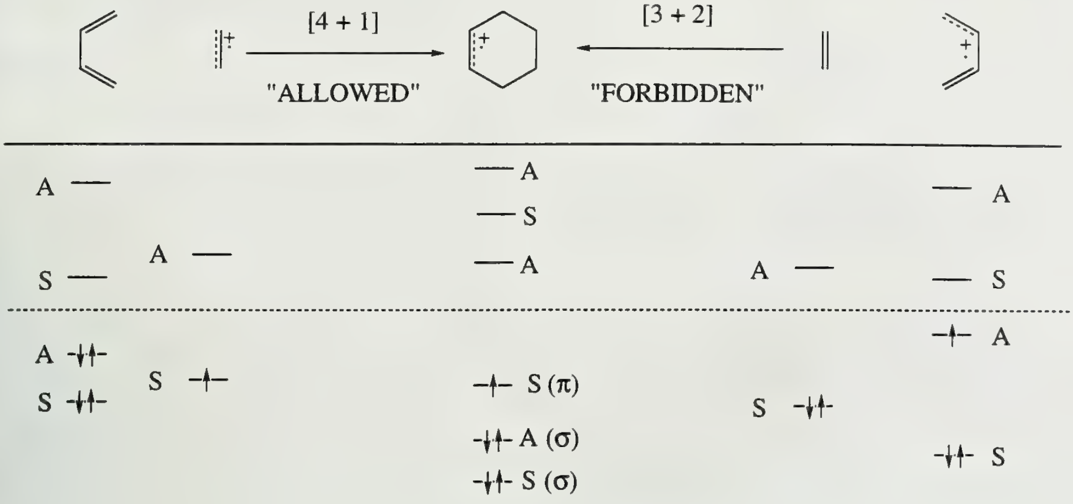
Scheme IV



EA: Electron Acceptor
CIP: Contact radical ion pair
SSIP: Solvent separated radical ion pair
R = H, OMe, OAc
s: Solvent

Early work based on orbital symmetry correlation suggested that only the addition of the radical cation dienophile to the neutral diene [4 + 1] is allowed while the addition of neutral dienophile to radical cation diene [3 + 2] is forbidden (Scheme V). Further studies demonstrated that the [3 + 2] process is also possible.[9,19]

Scheme V



The impressive facial selectivity (*endo* vs. *exo*) can be explained in terms of the enhancement of the secondary orbital interactions. The chemo- and regioselectivity of some of the radical cation D-A reactions can be explained by the calculated charge distribution, steric effects and stabilization of the transition state.[4]

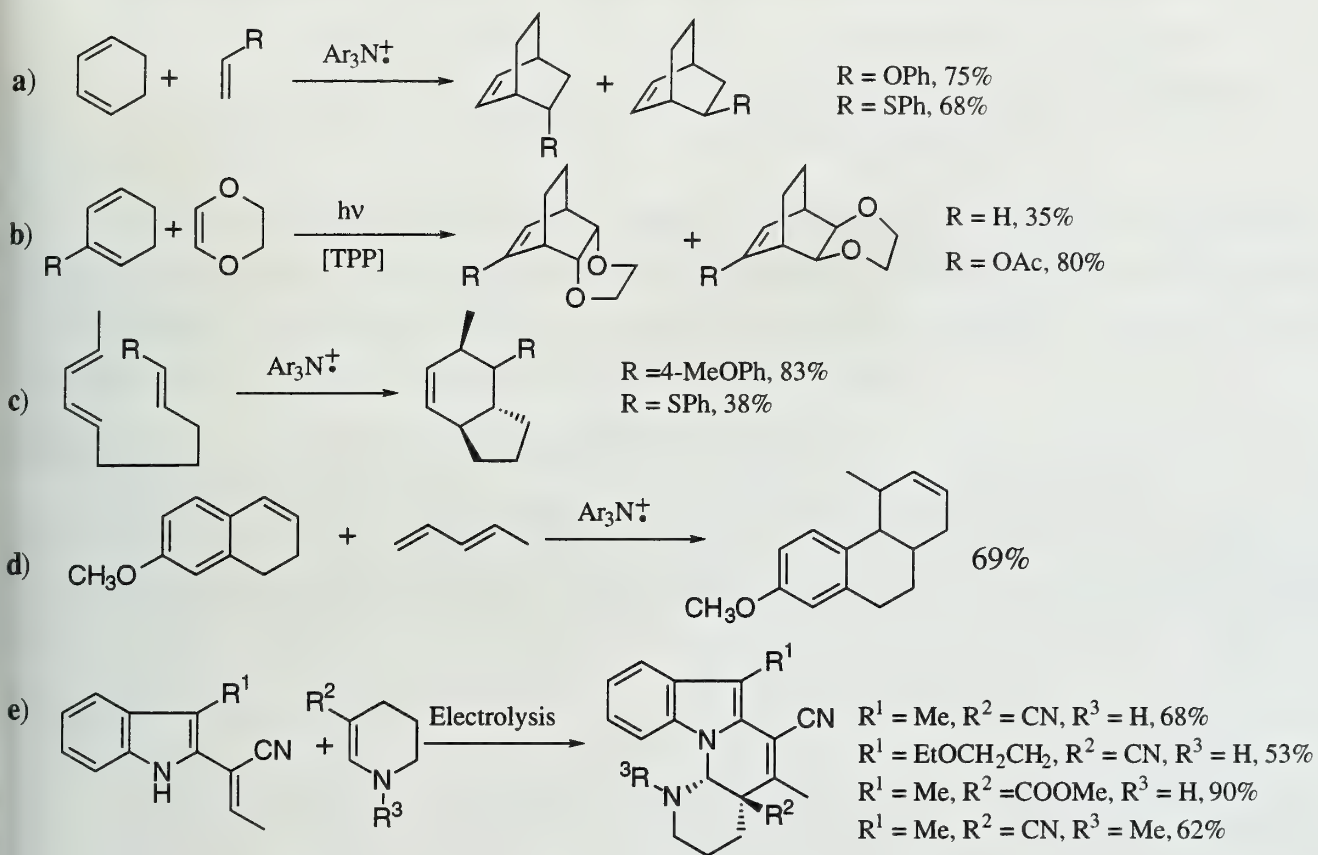
Synthetic Applications

Possible synthetic applications of the radical cation D-A reaction are apparent, however examples are extremely rare up to now. The reaction shown in Scheme I is the only example where the radical cation D-A reaction has been used in natural product synthesis [(-)-β-selinene].[1]

The most important radical cation D-A reactions might be those between electron rich dienophiles and dienes for which the neutral D-A does not work well. The mostly widely used diene component so far is 1,3-cyclohexadiene and its derivatives. Some reactions between 1,3-cyclohexadiene or its derivatives and electron rich dienophiles are listed as entries **a** and **b** of Scheme VI.[8] Other diene components that have been employed are 1-vinylcyclohexene[1], vinylindole derivatives,[20,21] exocyclic dienes[18], 1,3-cyclopentadiene[2], and 1,1'-dicyclohexenyl[22]. The dienophile component may be a conjugated diene[2] or electron rich olefins with substituted groups which can stabilize radical cations. Examples of electron rich olefins are: styrene and its electron rich derivatives[22,23], phenylvinylsulfide[1,23], ethylvinylether[23], vinylphenol[23], and indole[18,24] or its derivatives[18,25]. The radical cation D-A reactions between some electron-rich styrene derivatives and conjugated dienes have some possible use in the construction of steroidal hormones (entry **d**, Scheme VI).[23]

Some heterocycles have also been constructed with the radical cation D-A reactions. The D-A reaction between *N*-benzylideneaniline and an electron-rich vinyl ether to give tetrahydroquinolines is effected by the catalysis of K10 acidic montmorillonite doped with Fe[III].[26] The radical cation D-A reaction with vinyl indole derivatives as the diene component has demonstrated the ability to construct the skeleton of some alkaloids (entry **e**, Scheme VI).[21] Triplet O_2 was also found to react with certain conjugated dienes to give endoperoxides.[14,22]

Scheme VI



Fused rings attached to aromatic rings can be made through the radical cation D-A reaction using phenylated olefins as the diene component.[27] Fused ring systems were also constructed by intramolecular radical cation D-A reactions(entry **c**, Scheme VI).[11]

The fact that cycloadditions of ketenes and dienes under thermal conditions result in the formation of [2 + 2] cycloadducts instead of D-A products is well known. However, under radical cation conditions, this reaction has been successfully steered to the D-A reaction.[28,29]

A limitation of the radical cation D-A that both the diene and the dienophile need to be sufficiently electron-rich. This is important for both the single electron transfer and stabilization of the radical cation intermediates. In order for the reaction to be effective, the redox potential of the two species should be comparable($\Delta E_0 < 500$ mV, e.g.).[30] Dimerization of the diene component can be a major side reaction in the cross radical cation D-A and extra amount of dienophile is usually needed for

this reason.[23] Owing to the highly reactive nature of radical cations, some functional groups (-OH, -NH_3, e.g.) might not be compatible with the reaction.

RADICAL CATION CYCLOBUTANATION

Cyclobutanation between olefins can also be effected under radical cation generating conditions: through aminium salts or PET. Like radical cation D-A, both dimerizations and cross additions are possible during cyclobutanation. Electron rich alkenes are preferred because they are easy to oxidize.

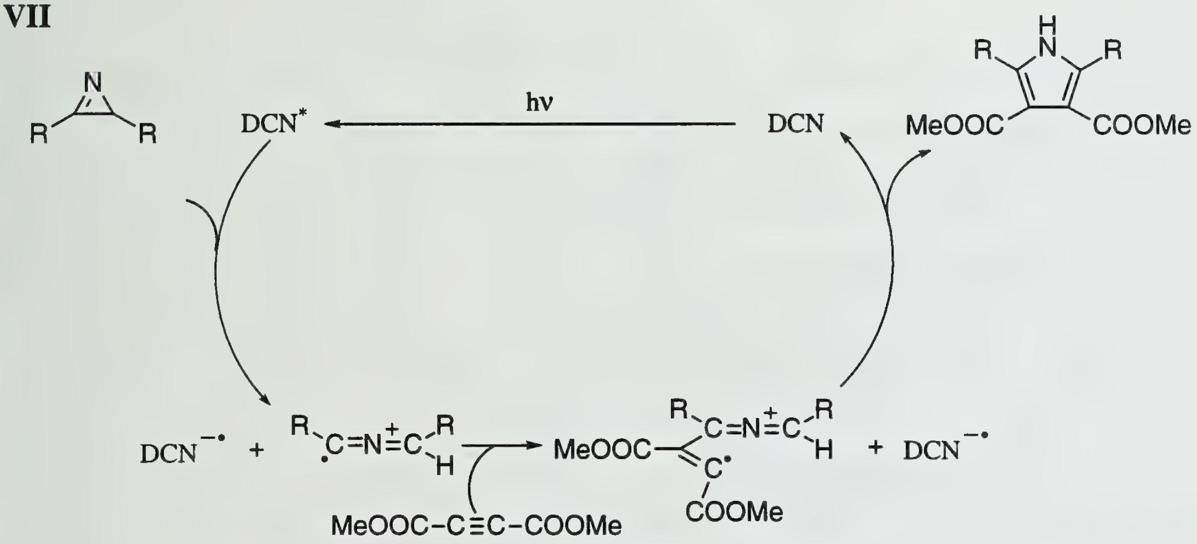
Cyclodimerization of either the *cis*- or the *trans*-anethole initiated by an aminium salt yield only cyclobutane derivatives with retention of configuration. This suggests that the radical cation cyclobutanation can be stereospecific. More recently, the stereoselectivity of the cyclobutanation was tested on a 2,6-diarylocta-1,6-diene system. The results support a stepwise but potentially selective mechanism.[31]

In addition to the construction of 4-membered ring systems, cyclobutanation also provides an indirect way to construct cyclohexene ring systems. In some of the radical cation cycloadditions between alkenes and conjugated dienes, the formation of vinylcyclobutanes is prefered.[32] These vinylcyclobutanes can be transformed easily to cyclohexene structures.[3]

OTHER RADICAL CATION CYCLOADDITION REACTION

Under PET conditions, ring opening of oxiranes[7,8], aziridines,[7,8,33-35] cyclopropanes[7,8], and thiiranes[7,8,36] occurs. The resulting radical cations react with olefin or to triplet oxygen to form five-membered rings. Electron donating groups in the three-membered rings are generally essential for high yields.[8]

Scheme VII



PET initiated radical cycloadditions with azirines have been succefully used in the construction of five-membered *N*-containing heterocycles.[34] Shown in Scheme VII is a proposed mechanism for the synthesis of pyrrole rings from azirines and dimethy 2-butynedioate. This reaction has been used in the synthesis of a porphyrin compound.[35]

CONCLUSION

A single electron transfer from an alkene causes significant changes in its properties. The resulting radical cation cycloaddition can make available a variety of cycloadducts which otherwise might be difficult to prepare. Good stereo- and regioselectivity can be achieved in radical cation cycloadditions. Depending on the nature of the reactants and the reaction conditions, different species in the charge transfer process might be involved in cycloadditions. A lot of theoretical work has been done and more experimental evidence is needed to reveal the mechanism of the reaction unambiguously. Synthetic applications are promising and remain to be explored further.

REFERENCES

(1) Harirchian, B.; Bauld, N. L. *J. Am. Chem. Soc.* **1989**, *111*, 1826.
(2) Bellville, D. J.; Bauld, N. L. *J. Am. Chem. Soc.* **1981**, *103*, 718.
(3) Bauld, N. L. *Tetrahedron* **1989**, *45*, 5307.
(4) Bellville, D. J.; Bauld, N. L.; Pabon, R. A.; Gardner, S. A. *J. Am. Chem. Soc.* **1983**, *105*, 3584.
(5) Schutte, R.; Freeman, G. R. *J. Am. Chem. Soc.* **1969**, *91*, 3715.
(6) Bauld, N. L.; Bellville, D. J.; Cogswell, G. *Tetrahedron Lett.* **1982**, *23*, 825.
(7) Mattay, J. *Synthesis* **1989**, 233.
(8) Müller, F.; Mattay, J. *Chem. Rev.* **1993**, *93*, 99.
(9) Mlcoch, J.; Steckhan, E. *Tetrahedron Lett.* **1987**, *28*, 1081.
(10) Al-Ekabi, H.; de Mayo, P. *J. Org. Chem.* **1987**, *52*, 4756.
(11) Bauld, N. L.; Bellville, D. J.; Harirchian, B.; Lorenz, K. T.; Pabon, R. L.; Reynolds, D. W.; Wirth, D. D.; Chiou, H.; Marsh, B. K. *Acc. Chem. Res.* **1987**, *20*, 371.
(12) Lorenz, K. T.; Bauld, N. L. *J. Am. Chem. Soc.* **1987**, *109*, 1157.
(13) Mattay, J.; Trampe, G.; Runsink, J. *Chem. Ber.* **1988**, *121*, 1991.
(14) Tang, R.; Yue, H. J.; Wolf, J. F.; Mares, F. *J. Am. Chem. Soc.* **1978**, *100*, 5248.
(15) Bellville, D. J.; Bauld, N. L. *Tetrahedron* **1986**, *42*, 6167.
(16) Bauld, N. L.; Bellville, D. J.; Pabon, R. A.; Chelsky, R.; Green, G. *J. Am. Chem. Soc.* **1983**, *105*, 2378.
(17) Wiest, O.; Steckhan, E.; Grein, F. *J. Org. Chem.* **1992**, *57*, 4034.
(18) Wiest, O.; Steckhan, E. *Tetrahedron Lett.* **1993**, *34*, 6391.
(19) Chockalingam, K.; Pinto, M.; Bauld, N. L. *J. Am. Chem. Soc.* **1990**, *112*, 447.
(20) Wiest, O.; Steckhan, E. *Angew. Chem. Int. Engl.* **1993**, *32*, 901.
(21) Gürtler, C. F.; Blechert, S.; Steckhan, E. *Angew. Chem. Int. Engl.* **1995**, *34*, 1900.
(22) Martiny, M.; Steckhan, E.; Esch, T. *Chem. Ber.* **1993**, *126*, 1671.
(23) Pabon, R. A.; Bellville, D. J.; Bauld, N. L. *J. Am. Chem. Soc.* **1983**, *105*, 5158.
(24) Gieseler, A.; Steckhan, E.; Wiest, O. *Synlett* **1990**, 275.
(25) Gieseler, A.; Steckhan, E.; Wiest, O.; Knoch, F. *J. Org. Chem.* **1991**, *56*, 1405.
(26) Cabral, J.; Laszlo, P.; Montaufier, M. T. *Tetrahedron Lett.* **1988**, *29*, 547.
(27) Maroulis, A. J.; Arnold, D. *J. Chem. Soc., Chem. Commun.* **1979**, 351.
(28) Schmittel, M.; von Seggern, H. *Angew. Chem. Int. Engl.* **1991**, *30*, 999.
(29) Schmittel, M.; von Seggern, H. *J. Am. Chem. Soc.* **1993**, *115*, 2165.
(30) Mlcoch, J.; Steckhan, E. *Angew. Chem. Int. Engl.* **1985**, *24*, 412.
(31) Takahashi, Y.; Ando, M.; Miyashi, T. *J. Chem. Soc., Chem. Commun.* **1995**, 521.
(32) Pabon, R. A.; Bellvill, D. J.; Bauld, N. L. *J. Am. Chem. Soc.* **1984**, *106*, 2730.
(33) Averdung, J.; Albrecht, E.; Lauterwein, J.; Luftmann, H.; Mattay, J.; Mohn, H.; Müller, W. H.; ter Meer, H. *Chem. Ber.* **1994**, *127*, 787.
(34) Müller, F.; Mattay, J.; Steenken, S. *J. Org. Chem.* **1993**, *58*, 4462.
(35) Müller, F.; Karwe, A.; Mattay, J. *J. Org. Chem.* **1992**, *57*, 6080.
(36) Glass, R. S.; Jung, W. *J. Am. Chem. Soc.* **1994**, *116*, 1137.

UNIVERSITY OF ILLINOIS-URBANA
Q.547IL6S C001
ORGANIC SEMINAR ABSTRACTS URBANA
1995-96:1
3 0112 025513950

Q.547
I16s
1995-96:2
CHX

ORGANIC SEMINAR ABSTRACTS
1995-96, SEMESTER II

University of Illinois

Department of Chemistry
Box 68 Roger Adams Laboratory
600 South Matthews Avenue
Urbana, Illinois 61801

June, 1996

Copyright © by the Board of Trustees of the University of Illinois

LIBRARY U. OF I., URBANA-CHAMPAIGN

NOTICE: Return or renew all Library Materials! The *Minimum* Fee for each Lost Book is $50.00.

The person charging this material is responsible for its return to the library from which it was withdrawn on or before the **Latest Date** stamped below.

Theft, mutilation, and underlining of books are reasons for disciplinary action and may result in dismissal from the University.
To renew call Telephone Center, 333-8400

UNIVERSITY OF ILLINOIS LIBRARY AT URBANA-CHAMPAIGN

CHEMISTRY LIBRARY

FEB 01 2001
OCT 16 REC'D

L161—O-1096

SEMINAR TOPICS

Semester II, 1995-96

Page

Phorbol Esters: Structure Activity Relationships with Protein Kinase C.................1
Matthew M. Ravn

Catalytic Asymmetric Hydrosilylation of Olefins Mediated by Chiral Phosphine-Palladium Complexes..9
Michael S. Terrazas

Artificial Ion Channels..17
Yue Zou

Systematic Stereochemical Determination of Polyene Macrolide Antibiotics: Determination of the Stereochemistry of Roflamycoin and Filipin........................25
R. Bruce Diebold

Preparation and Synthetic Utility of Vicinal Tricarbonyl Compounds 33
Qingwu Alan Jin

Inhibition of Epidermal Growth Factor Receptor Tyrosine Kinase (EGF-RTK)........41
Wang Mao

Synthesis and Activity of the Antifeedant: Azadirachtin 49
Brice C. Nelson

Additions to Carbonyl Groups in Aqueous Media...57
Timothy R. Wharton

Ligation Strategies in Protein Synthesis..65
Lisa Flugge

Evolution's Middle-Age: Chemical Progenitors of Life..................................73
Brian Hubbard

Phorbol Esters: Structure Activity Relationships With Protein Kinase C

Reported by Matthew M. Ravn February 15, 1996

Introduction

Since its discovery by Nishizuka in 1977,[1] protein kinase C (PKC) has attracted considerable attention due to its key role in signal transduction pathways. This family of serine/threonine protein kinases is involved in one of the two branches of the message transduction activated by phosphatidylinositol 4-5-bis-phosphate turnover. PKC's activation by growth factors, neurotransmitters, and hormones has been thoroughly studied,[2] and its involvement in cell differentiation makes members of this family attractive targets for chemotherapeutic agents.

A number of structurally diverse natural products act as ultrapotent activators of the PKC enzyme. These include phorbol esters, teleocidins, ingenols and aplysiatoxins. The size and characteristics of PKC have prevented direct structural characterization by NMR or crystallographic methods. Studies of PKC binding sites have instead centered around other known methods of binding site determination such as structure activity relationships of PKC's binding site, molecular modeling, synthetic analogs and photoaffinity studies. Elucidation of the structural requirements necessary for biological activity would offer important insights into the design of therapeutic agents that specifically target PKC's. Site directed mutagenesis has also been performed but will not be discussed.

In response to specific extracellular signals, phosphatidylinositol 4-5-bis-phosphate is hydrolyzed to produce *syn* -1,2-diacylglycerols (DAGs) and inositol 1,4,5-triphosphate, two cytosolic secondary messengers.[3] DAGs are transient activators of the PKC enzyme which induce binding of the enzyme to the lipid bilayer. The current model of activation assumes that translocation of PKC to the lipid bilayer induces a conformational change that results in a catalytically active enzyme capable of phosphorylating a large number of cellular proteins.[4,5] This is followed by rapid inactivation of the enzyme, presumably by metabolism of the DAGs. In the case of phorbol ester binding, the only available pathway for deactivation of the PKC/lipid complex is proteolytic degradation of the protein.[6,7]

To date, eleven isozymes of PKC have been identified and classified into three groups based on DAG/phorbol ester binding and co-factor requirements.[5,9-11] PKCs requires zinc and phosphatidylserine as binding substrates, and many of the isozymes require calcium for proper function of the catalytic domain. In general, the protein is composed of two domains, a 50 kDa catalytic domain at the carboxyl terminus which is responsible for the activity of the kinase, and a 32

Copyright © 1996 by Matthew M. Ravn

kDa regulatory domain at the amino terminus. Most of the isozymes can be further characterized as having four homologous (C_1- C_4) and five variable (V_1-V_5) regions. The binding domains have been shown to consist of two cystine rich regions in the first homologous domain of the regulatory subunit. Referred to as cys1 and cys2, these regions are approximately 50 amino acid in size and retain binding affinities comparable to natural PKC.[12,13] These protein fragments are often utilized in place of the full PKC protein for binding studies.

Structure-Activity Relationships

Phorbol esters are highly functionalized tetracyclic tiglane diterpenoids. The medicinal plant *Croton tiglium* is the source of 12-*O* -tetradecanoylphorbol 13-acetate (TPA), a potent tumor promoter of this class of natural products.[14] The biologically active phorbol esters can be dissected in to a hydrophilic and a hydrophobic domain. The hydrophilic region occupies the five and seven membered rings on one face of the molecule between C-3 and C-9. The other portion of the ester, which is comprised of the six and three membered rings with acyl units on the C-12 and C-13 positions, constitutes the hydrophobic region.

Table I

Compound	Activity*	Compound	Activity*
TPA	++++	PMA-6-dehydroxymethyl	None
PDD (12,13-didecanoate)	++++	TPA-20-acetate	+++
PDBu (12,13-dibutyrate)	++++	TPA-20-tetradeconate	+
12-0-tetradecanoylphorbol	None	TPA-20-aldehyde	+
Phorbol	None	TPA-20-acid	None
TPA-6β,7β-oxide	+++	TPA-4β-deoxy	+++
TPA-6α,7α-oxide	+++	TPA-3β-ol	++
TPA-1,2-oxide	None	PDD-4α	None

*Activity represents complete or second-stage tumor promoting activity on mouse skin. Standard procedure, NMRI mice.[18] Data compiled form various sources.[14-17]

Structure-activity relationships must be interpreted with caution since tumor promotion is not necessarily a function of binding affinities. These structure-activity relationships suggest the following features are necessary for biological activity and/or binding: i) the hydrophobic acyl group(s) on C-12 and/or C-13, ii) a hydrophilic group at C-20 that can preferentially act as a hydrogen bond donor and acceptor iii) the keto group at C-3 and/or hydroxyl group at C-4 and iv) a $\Delta^{1,2}$ bond.

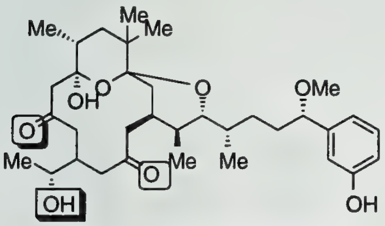
Modeling Studies

In cases such as PKC where a number of diverse chemical structures are known to bind to the same active site, molecular modeling is an especially useful tool. A rapid progression of models during the late 1980's and early 1990's resulted from the intense application of this methodology.[16-23] The general approach utilized by all studies was to prepare minimized structures of the compounds that activate PKC and relate their spatial coordinates or characteristics to each other. In the case of non-rigid molecules such as teleocidins, local minima were also considered if they did not exceed the global minima by 5-6 kcal/mol. Structure-activity relationships of the compounds utilized in the modeling studies were also considered to explain the modeling results.

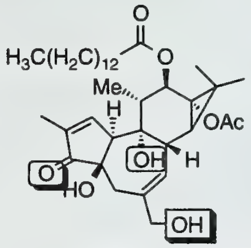
Regardless of the range of compounds and structural correlations utilized, a number of common features emerged. The requirement for the hydroxyl group at C-20 and the need for a hydrophobic moiety in the C-12/C-13 region were universally agreed upon. The keto group at C-3 and the hydroxyl group at C-4 were used interchangeably. Most studies considered only one of these moieties important and proposed that the other could serve as a surrogate in the absence of the first. The C-9 hydroxyl group was also considered necessary in five of the six studies.

Rando and Kishi have produced a new model based on advances in the structure-activity relationships of aplysiatoxins. This model proposes that the C-3 keto and the C-9 and C-20 hydroxy oxygens are necessary. (Figure 1)

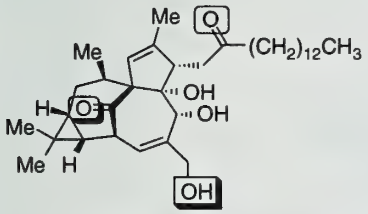
Figure 1



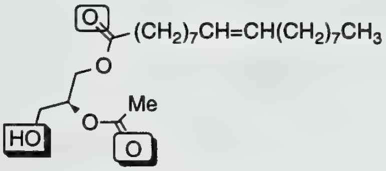
Debromoaplysiatoxin (DAT) **1**



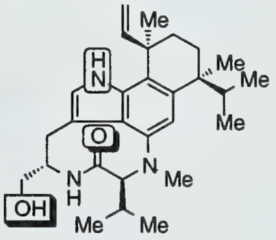
12-*O*-tetradecanoylphorbol-13-acetate (TPA) **2**



Ingenol-3-O-tetradecanoate **3**



(S)-1-Oleoyl-2-acetylglycerol **4**



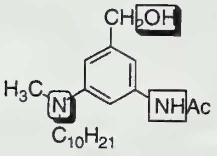
Teleocidin B-1 **5**

Synthetic Analogs

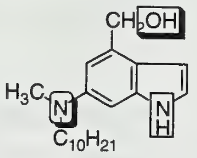
Two studies have tested the proposed model of activity of the phorbol esters. Wender *et. al*[20] synthesized simple molecules which possessed functional groups at the correct positions to match the

C-4,C-9,C-20 hydroxyl binding model. The aniline and indole analogs were prepared using known methods, and by the Leimgruber method for synthesis of the indole core.[24] These derivatives showed binding to PKCs at 20 to 30,000 times lower affinity than TPA and 10 times lower affinity than DAGs. This marks the first instance where a synthetic product prepared through rational design interacted strongly with PKC.

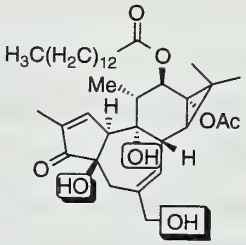
Figure 2



ADMB 6



DHMI 7

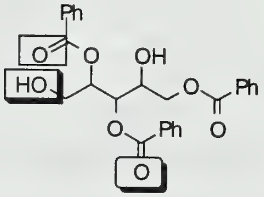


TPA 2

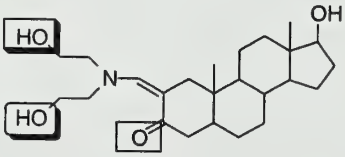
Compound	K_i
6	22 µM
7	12 µM
2	1.3 nM

A database search based on the C-3,C-9,C-20 model proposed by Kishi and Rando produced the second group of synthetic analogs.[25] Due to the ability to produce new leads in drug discovery programs, three dimensional database searches for potential pharmacophore structures are becoming more popular.[26,27] Chem-X[28] was utilized to generate a 3D database of 407,000 structures from the 2D structures of the NCI Drug Information System database.[29] A search query consisting of the C-3,C-9,C-20 model generated 535 hits, of which 286 were available for immediate testing. The lack of a hydrophobic region around C-12/C-13 eliminated 161 of these compounds. Upon assaying of 125 remaining compounds, five new significantly active compounds were found. As with the Wender analogs, these new classes bound PKCα with similar affinities as DAGs.

Figure 3

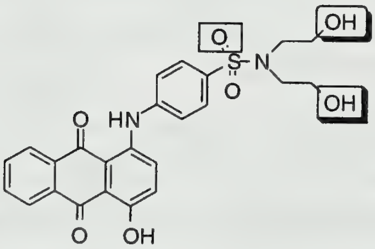


8

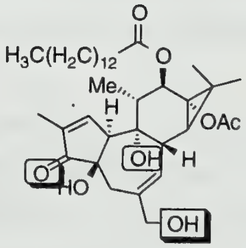


9

Compound	K_i
8	13 µM
9	16 µM
10	8 µM
2	1.3 nM



10



TPA 2

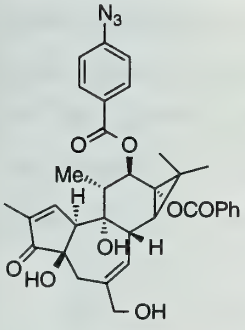
Photoaffinity Studies

A standard method for the determination of spatial relationships in protein/ligand complexes for which 3-D structural data is unavailable is the use of photoaffinity derivatives. Photoaffinity experiments using phorbol esters have been limited due in part to the difficulty associated with selective functional group manipulation. In addition, photoaffinity derivatives which still possess binding affinity for a PKC protein have been difficult to develop. To date, only three significantly different yet viable photoaffinity derivatives have been synthesized.[30-33]

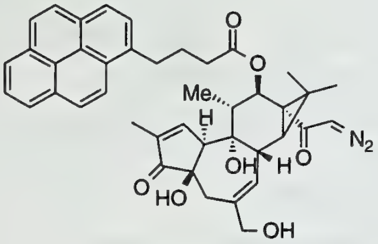
Blumberg and Hecker [30,31] have both developed photoaffinity derivatives at the C-12 acyl position. The [20-^{3}H]-12-p-azidobenzoylphorbol-13-benzoate (**11**) and the [20-^{3}H]-12-(5-azido-2-nitro)benzoylphorbol 13-acetate were tested *in vivo* and found to preferentially label the phosphatidylserine lipid cofactor. Dark test runs demonstrated that the binding constants were essentially the same as the binding affinities observed for PDBu.

The photoaffinity derivative, [20-^{3}H]-phorbol 12-(1-pyrenebutyrate) 13-diazoacetate (PPDA), was tested *in vitro* on natural PKCs and found to crosslink with the protein based on SDS-PAGE assays.[32] Dark test runs confirmed that this derivative was comparable to PMA in binding affinity. To date, this is the only known photoaffinity ligand prepared which crosslinks to the protein. A more detailed analysis to determine which amino acids were crosslinked has yet to be reported.

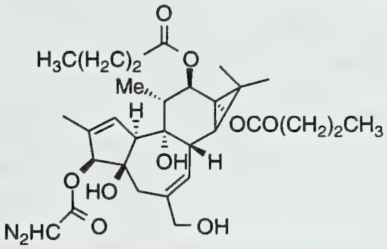
Figure 4



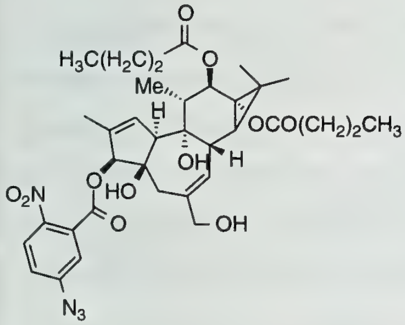
11



12



13



14

Compound	K	Labeling
11	1 nM (K_d)	Phospatidylserine
12	20 nM (IC_{50})	PKC
13	693 nM (IC_{50})	ND
14	46 nM (IC_{50})	ND

Wender *et. al* have prepared (5-azido-2-nitro)benzoyl (**14**) and diazoacetate (**13**) photoaffinity derivatives at the C-3 position.[33] While dark run affinity testing showed that the affinities are 100 and 10 fold weaker than PDBu, they may still function as photoaffinity probes. Experimental data on the labeling of PKC has not been reported. Instead, the solvolytic fate of the nitrene or carbene intermediates were tested. The diazoacetate provided preferential solvent trapping (54%), in addition to other byproducts. By contrast, the azido derivative did not provide encouraging results, giving a number of unidentified degradation products.

Crystal Structure

Crystal structures of the cys2 domain of PKCγ in the presence of phorbol-13-acetate have been refined to an R factor of 19.4% at 2.2Å resolution.[34] In the phorbol/protein complex, hydrogen bonds were assigned between Gly-253 with C-3 and C-4 oxygens, and Tyr-242 and Leu-251 with the C-20 hydroxyl. Surprisingly, the C-9 hydroxyl did not participate in a hydrogen bond to the protein, but instead participated in an intermolecular hydrogen bond with the C-13 acetyl group. Recall that most modeling studies identified the C-9 hydroxyl as a necessary feature. The binding of the phorbol ester in a hydrophilic cleft produced a continuous hydrophobic region. This would allow the insertion or association of the protein/phorbol complex with the lipid bilayer, consistant with the models.

While heralded as a major advance in the understanding of PKC/phorbol ester binding, there exist reasons to be cautious.[35] For example, the phorbol-13-acetate ligand chosen has not been demonstrated to be an activator of PKC. Its use was a compromise to promote solubility of the phorbol ester as well as the protein so that crystallization would be possible. Furthermore, phosphatidylserine is a necessary cofactor required for binding in solution, so a structure which lacks this cofactor is not necessarily complete.

Conclusions

While many advances and important discoveries have been made during the past two decades, a number of questions remain regarding the phorbol ester pharmacophore. Among the most relevant questions are the roles of phosphatidylserine and the C-9 hydroxyl group in PKC phorbol binding. Preliminary results from Wender's lab indicate that the phospholipid induces conformational rigidity in cys1 and cys2 protein fragments.[36] Further studies are underway on the nature of the quaternary phospholipid/peptide structure. Recent advances in the total synthesis of phorbol esters[37,38] might permit the experimental elucidation of the role of the C-9 hydroxyl.

References

1) Takai, Y.; Kishimoto, A.; Inoue, M.; Nishizuka, Y. *J. Biol. Chem.* **1977**, *252*, 7603.

2) For a review, see: *Protien Kinase C. Current Concepts and Future Perspectives*; Lester, D. S.; Epand R.M. Eds.; Horwood:New York, 1992.

3) a) Hokin, L. E. *Annu. Rev. Biochem.* **1985**, 54, 205. b) Berridge, M. J. *Annu. Rev. Biochem.* **1987**, *56*, 159.

4) Nishizuka, Y. *Science* **1986**, *233*, 305.

5) Nishizuka, Y. *Nature (London)* **1988**, *334*, 661.

6) Kishimoto, A.; Mikawa, K.; Hashimoto, K.; Yasuda, I.; Tanaka, S.; Tominaga, M.; Kuroda, T.; Nishizuka, Y. *J. Biol. Chem.* **1989**, *264*, 4088.

7) Junco, M.; Webster, C.; Crawford, C.; Bosca, L.; Parker, P.J. *Eur. J. Bioichem.* **1994**, *223*, 259.

8) Nishizuka, Y. *Nature (London)*, **1984**, *308*, 693. and references therein

9) Osada, S.; Mizuno, K.; Saido, T. C.; Akita, Y.; Suzuki, K.; Kuroki, T.; Ohno, S. *J. Biol. Chem.* **1990**, *265*, 22434.

10) Bacher, N.; Zisman, Y.; Berent, E.; Livneh, E. *Mol. Cell. Biol.* **1991**, *11*, 126.

11) Ono, Y.; Fujii, T.; Ogita, K.; Kikkawa, U.; Igarashi, K.; Nishizuka, Y. *J. Biol. Chem.* **1988**, *263*, 6927.

12) Quest, A. F. G.; Bardes, E. S. G.; Bell, R. M. *J. Biol. Chem.* **1994**, *296*, 2953.

13) Quest, A. F. G.; Bardes, E. S. G.; Bell, R. M. *J. Biol. Chem.* **1994**, *296*, 2961.

14) Hecker, E. in *Carcinogenesis: Mechanisms of Tumor Promotion and Cocarcinogenesis* ; Slaga, T. J.; Sivak, A; Boutwell, R. K. Eds.; Raven: New York, 1978; Vol. 2, pp. 11-48.

5) Weinstein, I. B.; Arcoleo, J.; Backer, J.; Jeffery, A.; Hsiao. W. W.; Gattoni-Celli, S.; Kirshmeier, P.; Okin, E. in *Cellular Interactions by Enviromental Tumor Promoters*; Fujiki, H.; Hecker, E.; Morre, R. E.; Sugimura, T. Eds.; Japan Science Society Press:Tokyo; pp. 59-74.

6) Jeffery, A. M.; Liskamp, R. M. *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 241.

7) Sugita, K.; Neville, C. F.; Sodeoka, M.; Sasai, H.; Masakatsu, S. *Tet. Lett.* **1995**, *36*, 1067.

8) Hecker, E.; Schmidt R. in *Methods of Cancer Research*; Busch, H. Ed.; Academic Press:New York; Vol. 6, pp 439-484 .

9) Wender, P. A.; Koehler, K. F.; Sharkey, N. A.; Dell'Aquila, M. L.; Blumberg, P. M. *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 4214.

0) Wender, P. A.; Cribbs, C. M.; Koehler, K. F.; Sharkey, N. A.; Herald, C. L.; Kamano, Y.; Pettit, G. R.; Blumberg, P. M. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 7197.

1) Itai, A.; Kato, Y.; Tomioka, N.; Iitaka, Y.; Endo, Y.; Hasegawa, M.; Shudo, K.; Fujiki, H.; Sakai, S. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 3688.

2) Nakamura, H.; Kishi, Y.; Pajares, M. A.; Rando, R. R. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 9672.

3) Kishi, Y.; Rando, R. R. *Biochemisty* **1992**, *31*, 2211.

24) Batcho, A. D.; Leimgruber, W. *Org. Synth.* **1984**, *63*, 214.

25) Wang, S.; Zaharevitz, D. W.; Sharma, R.; Marquez, V. E.; Lewin, N. E.; Du, L.; Blumberg, P. M.; Milne, G. W. A. *J. Med. Chem.* **1994**, *37*, 4479.

26) (a) Martin, Y. C. *J. Med. Chem.* **1992**, *35*, 2145. (b) Martin, Y. C.; Bures, M. G.; Willett, P. *Reviews in Computational Chemistry*; Lipkowitz, K. B.; Boyd, D. B. Eds.; VCH Publishers:New York, 1990; pp 213-263.

27) Sheridan, R. P.; Rusinko, A. III; Nilakantan, R.; Venkataraghavan, R. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 8165.

28) Chem-X is a product of Chemical Design Ltd., Roundway house, Cromwell Park, Chipping Norton, Oxfordshire OX7 5SR, England.

29) The NCI Drug Information System was designed and built by NCI. See Milne, G. W. A.; Miller, J. A.; et al. *J. Chem. Inf. Comput. Sci.* **1986**, *26*, 154.

30) Schmidt, R.; Heck, K.; Sorg, B.; Hecker, E. *Cancer Letters*, **1985**, *26*, 97.

31) Delcols, K. B.; Yeh, E.; Blumberg, P. M. *Proc Natl. Acad. Sci. U.S.A.* **1984**, *80*, 3054.

32) Sodeoka, M.; Uotsu, K.; Shibasaki, M. *Tet. Lett.* **1995**, *36*, 8795.

33) Wender, P. A.; Irie, K.; Millier, B. L. *J. Org. Chem.* **1993**, *58*, 4179.

34) Zhang, G.; Kazanietz, M. G.; Blumberg, P. M.; Hurley, J. H. *Cell* **1995**, *81*, 917.

35) Rouhi, M. A. *Chem. Eng. News* **1995**, 21.

36) Wender, P. A.; Kazuhiro, I.; Miller, B. L. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 239.

37) Wender, P. A.; McDonald, F. E. *J. Am. Chem. Soc.* **1990**, *112*, 4956.

38) Kazuyki S.; Kazuhiko, S.; Neville, C. F.; Sasai, H.; Shibasaki, M. *Synlett* **1994**, 325.

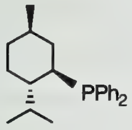
CATALYTIC ASYMMETRIC HYDROSILYLATION OF OLEFINS MEDIATED BY CHIRAL PHOSPHINE-PALLADIUM COMPLEXES

Reported by Michael S. Terrazas February 26, 1996

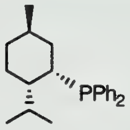
INTRODUCTION

Palladium catalysts were initially regarded as inactive toward hydrosilylation reactions since they are often reduced to the metallic state.[1] This view is incorrect as a variety of olefins have been hydrosilylated using palladium catalysts and often give products with regiochemistries and stereochemistries not available with other catalysts.[2] Despite some unique reactivities, little attention had been paid to the potential of palladium as a hydrosilylation reaction catalyst until it was discovered that alkylsilanes could be oxidized to alcohols. The methodology and mechanism of palladium-catalyzed asymmetric hydrosilylations will be the primary focus of this review.

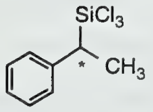
Hydrosilylation of acyclic olefins in the presence of platinum(II) complexes[3] is an efficient and frequently used method in industry. The reagent is generally regiospecific affording only terminal silanes. By contrast, palladium(II)-phosphine mediated hydrosilylation reactions often give Markovnikov products containing a stereogenic center at the carbon attached to the silicon. In addition, use of chiral phosphine ligands coordinated to palladium catalysts provides an environment that induces asymmetry in the products of hydrosilylation reactions. In 1972, Kumada and co-workers[4] reported the first asymmetric hydrosilylation of styrene with $HSiCl_3$ catalyzed by chiral phosphine-Pd(II) complexes. The palladium(II) chlorides of menthyldiphenylphosphine (MDPP) **1** and neomenthyldiphenylphosphine (NMDPP) **2** afforded the regiospecific α-phenylethyltrichlorosilane **3** in high yields and high catalytic activity. Enantioselectivities were very low (5% ee), but enough to spark interest in this area. New catalysts have since been designed that are more selective and can accomodate a wider range of substrates.



1



2



3

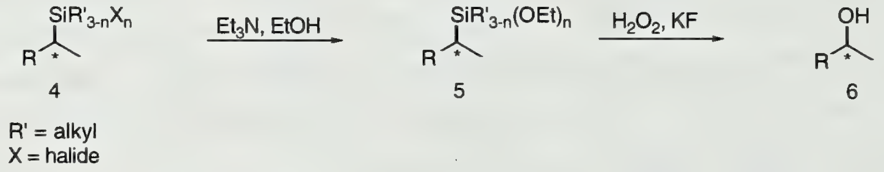
GENERAL CONDITIONS FOR CATALYTIC HYDROSILYLATION REACTIONS

Palladium-catalyzed hydrosilylation reactions are an attractive methodology for use in industrial applications because reactions can be run neat as most hydrosilanes are oils. Reaction conditions vary only in temperature and time with ranges of 0 °C to 80 °C and a few hours to a few days being typical. Many of the catalysts are prepared in situ by mixing the chiral phosphine ligand and a palladium(II) chloride complex in a 2:1 ratio. Usually, a mixture of substrate, one equivalent of hydrosilane, and

Copyright © 1996 by Michael S. Terrazas

catalyst (usually 0.1 mol% or less) are stirred in a sealed glass ampoule under argon. Most often Kugelrohr distillation of the crude mixture yields pure oganosilane.

The absolute configurations of the products obtained from asymmetric hydrosilylation reactions are determined by the conversion of the enantioenriched organosilane to the corresponding alcohol via a Tamoa oxidation (equation 1).[5] The chlorosilane **4** is first treated with Et_3N and EtOH to afford an ethoxysilane **5** which is subsequently transformed to the alcohol **6** by the oxidative cleavage of the carbon-silicon bond with hydrogen peroxide in the presence of potassium fluoride. The process is known to proceed with overall retention of configuration and high yields.



(1)

CATALYTIC CYCLE

The accepted mechanism for the palladium-catalyzed hydrosilylation of non-conjugated olefins (Figure 1) is based upon the hydrometallation mechanism proposed by Chalk and Harrod.[1,6] The active catalyst is believed to be a 16-electron palladium(0) complex formed from the in situ reduction of a palladium(II) chloride.[7] The reduction has been suggested to proceed by oxidative-insertion of hydrosilane followed by a double elimination of chlorosilane and HCl.[8] The catalytic cycle can be divided into four steps: *a*) coordination of the palladium(0) complex to an olefin forming a palladium(0)-π-complex; *b*) oxidative-insertion of the π-complex into the Si-H bond of the hydrosilane

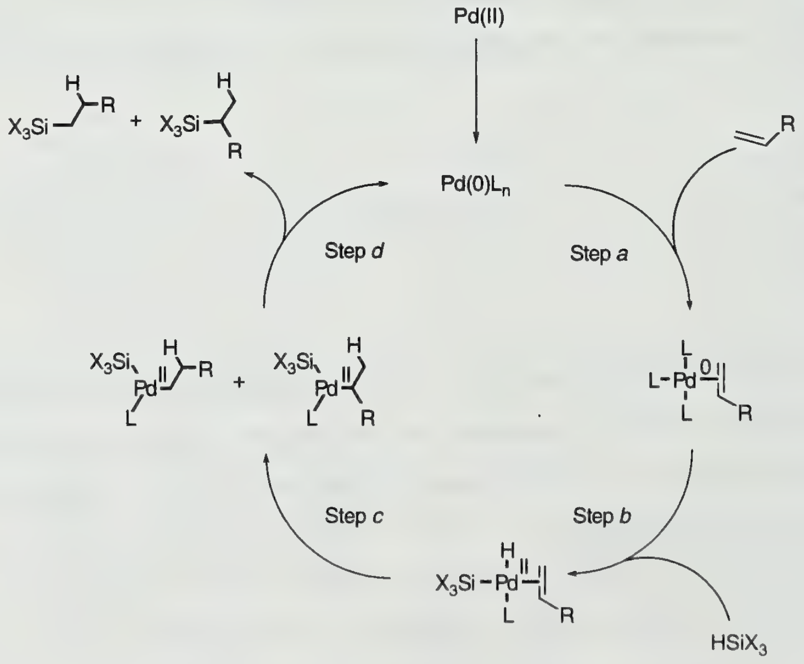


Figure 1. Catalytic cycle for the palladium-catalyzed hydrosilylation of olefins.

giving a hydrido(silyl)palladium(II) complex; *c*) migration of the hydride either of the olefinic carbons to afford a 14-electron unsaturated palladium(II) σ-complex; *d*) release of the organosilane by the reductive-elimination of the palladium(II) complex to palladium(0). The palladium-catalyzed hydrosilylation of 1,3-dienes occurs by a similar mechanism that involves a π-allyl complex as a key intermediate (*vida infra*).

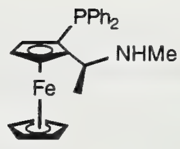
ROLE OF HYDROSILANE

Choice of hydrosilane used in palladium-catalyzed hydrosilylation reactions has a major effect on observed catalytic activities and yields.[7] The hydrosilane requires an electron-withdrawing group attached to the silicon in order for the catalyst to exhibit any activity. Electron-withdrawing groups strengthen the Pd-Si bond of the hydrido(silyl)palladium(II) complex increasing the facility of formation of the complex.[6] The general order of activity of hydrosilanes is: $HSiCl_3 > HSiMeCl_2 > HSiMe_2Cl > HSi(OR)_3 > HSiR_3$.[9]

Hatanaka and co-workers have reported that hydrosilanes also affect the observed enantioselectivity of palladium-catalyzed hydrosilylation reactions.[8] When diphenylfluorosilane was used for the hydrosilylation of (*E*)-1-phenyl-1,3-butadiene, the resultin silane was obtained with 66% ee as compared to the 34% ee observed with diphenylchlorosilane. However, fluorosilanes generally show lower catalytic activity as compared to chlorosilanes.[9] The reason for the observed differences in enantioselectivities and catalytic activities has not been established.

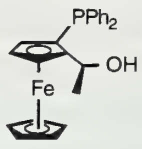
CHIRAL PHOSPHINE LIGANDS

Monodentate phosphines are the best ligands for palladium catalyzed hydrosilylation reactions. Many of the chiral phosphine ligands used in asymmetric reactions are chelating bisphosphines which coordinate the metal in a bidentate fashion to provide a chiral reaction environment.[10] Bisphosphines show little or no activity when used in hydrosilylation reactions. Hayashi reported that palladium complexes coordinated to 1,4-bis(diphenylphosphino)butane (dppb), (*S,S*)-2,3-bis(diphenylphosphino)butane (chiraphos), and (*R*)-2,2'-bis(diphenylphosphino)-1,1'-binaphthyl (BINAP) show a complete loss of catalytic activity for the hydrosilylation of 1-alkenes.[11] It was argued that a monodentate phosphine ligand leads to a more active square-planar palladium intermediate and offers a coordination site for the olefin.[11] Among the ligands which have shown moderate to high enantioselectivities when coordinated to palladium include chiral ferrocenylphosphines **8**,[8,12] *P*-menthylphosphetanes **9**[13] (β-*N*-sulfonylaminoalkyl)phosphine **10**[14] and binaphthylmonophosphines **11** (MOP).[11,15]



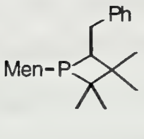
(*R*)-(*S*)-PPFA

8a

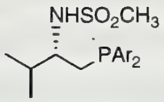


(*R*)-(*S*)-PPF-OH

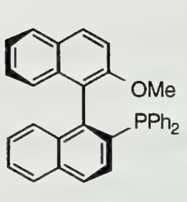
8b



9

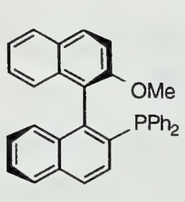


10



(*S*)-MOP

(*S*)-**11**



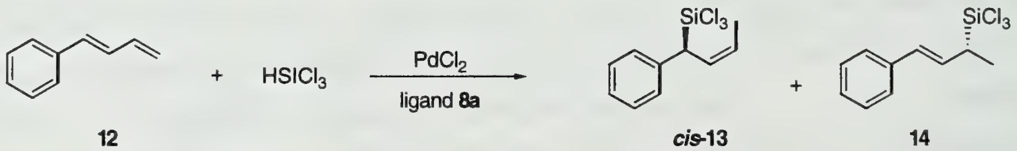
(*R*)-MOP

(*R*)-**11**

HYDROSILYLATION OF CYCLIC AND ACYCLIC 1,3-DIENES

Palladium-catalyzed asymmetric hydrosilylation of cyclic and acyclic conjugated dienes can produce enantioenriched allylsilanes.[8,13,14,17] These products are valuable reagents for carbon-carbon bond forming reactions via S_E' reactions.[16] Allylsilanes can also be converted into enantioenriched allylic alcohols by oxidation of the carbon-silicon bond with aqueous H_2O_2 with retention of configuration.[5] The best results have been observed with palladium(II)-ligand **8** complexes. While only moderate enantioselectivities have been obtained, regioselectivities and catalytic activities have been high. Moderate enantioselectivities have been detected upon the hydrosilylation of 1,3-cyclohexadiene (72% ee), 1-cyclohexyl-1,3-butadiene (52% ee), 1,3-heptadiene (37% ee), 1,3-pentadiene (69% ee), and 1,3-hexadiene (69% ee).

The palladium-catalyzed hydrosilylation of 1,3-dienes proceeds via a 1,4-addition of the hydrosilane. This contrasts with the platinum catalysts which favor the 1,2-addition of the hydrosilane to the diene. The reaction of 1-phenyl-1,3-butadiene (**12**) and trichlorosilane catalyzed by (*R*)-(*S*)-PPFA-Pd(II)Cl_2 affords two regioisomers, *cis*-1-phenyl-1-trichlorosilyl-2-butene (**13**) and *trans*-1-phenyl-3-trichlorosilyl-1-butene (**14**) in a 94:6 ratio (equation 2).



(2)

12

***cis*-13**
64% ee

14
30% ee

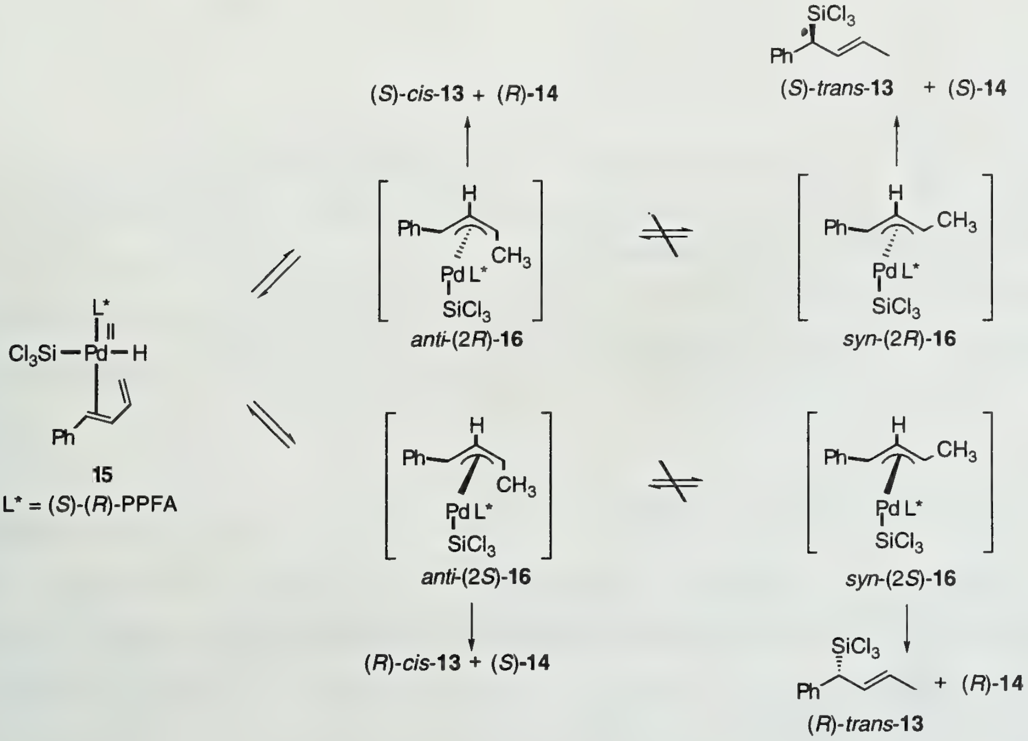
13:**14** = 94:6

The accepted mechanism for the hydrosilylation of 1,3-dienes (Scheme 1) has the same the catalytic cycle as shown for terminal alkenes in Figure 1. The only difference is that a π-allyl complex is formed as the key intermediate following hydride migration and not the Pd-C σ-complex.[17a] The palladium(II) complex coordinates with the diene in a cisoid conformation (**15**). Migration of the hydride to the terminal end of the diene affords the diastereomeric π-allyl complex **16**. This places the methyl group in an *anti*-conformation with respect to the center carbon-hydrogen bond of the allyl group. Silyl migration to either the 1- or 3- position of the allyl group by a reductive-elimination of palladium(II) π-allyl complex **16** affords regioisomers **13** and **14** respectively. NMR studies of palladium-π-allyl complexes by Trost et al.[17a] showed that alkyl substituents at either end of the allyl group prefer a *syn*-conformation. In principle, the *anti*-**16** diastereomer could undergo an isomerization to *syn*-**16** via a σ–

π–σ mechanism. However, the absence of *trans*-**13**, arising from *syn*-**16**, demonstrates that the silyl migration is faster than isomerization of the *anti*-**16** diastereomer.[19]

The ratio of regioisomers **13:14** is likely determined by the steric nature of the substituents at the 1- and 3- positions of the π-allyl intermediate **16**. Migration of the silyl group to a carbon containing an *anti* substituent is less able to occur than with a *syn* substituent during the reductive-elimination step.

Scheme 1



The determinant of enantioselectivity is unclear based on the results of the hydrosilylation of **12**. The observation of (*S*)-*cis*-**13** and (*R*)-**14** as the major enantiomers were taken to suggest that the stereochemistry is set by the preferential formation of the palladium-π-allyl complex *anti*-(2*R*)-**16**.[17a] However, no experimental evidence was advanced in support of this hypothesis.

An alternative explanation may be proposed assuming the reaction follows a path to which the Curtin-Hammett Principle may be applied. Product ratios would be determined by the difference in the diastereomeric transition state energy ($\Delta\Delta G^{\ddagger}$) leading to products and not by the relative concentration of the palladium-π-allyl intermediates. In this case the equilibration between *anti*-(2*R*)-**16** and *anti*-(2*S*)-**16** must be faster than the silyl transfer.

HYDROSILYLATION OF TERMINAL OLEFINS

The most significant advance in Pd-catalyzed asymmetric hydrosilylation reactions has been the use of MOP **11** for palladium-catalyzed hydrosilylation reactions of styrenes and terminal nonconjugated olefins.[20,21] A wide array of substrates, including 1-alkenes,[11] styrene,[15c] bicyclic olefins,[15a] and heterocyclic olefins,[22] were hydrosilylated in high yields and good enantioselectivies (Figure 2). The mechanism of asymmetric induction using the MOP ligand is unclear but must involve diastereomeric intermediates.

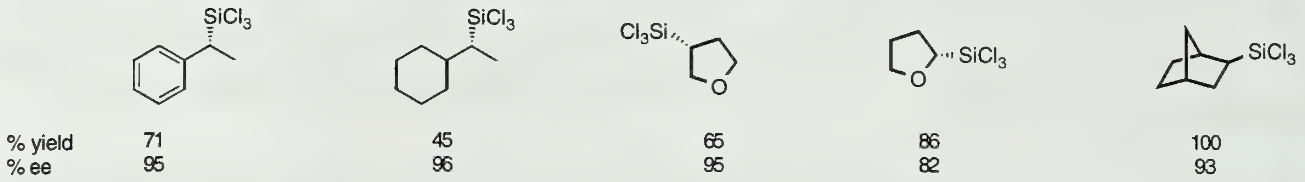
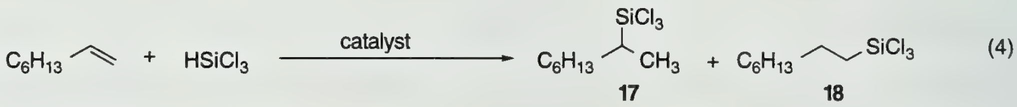


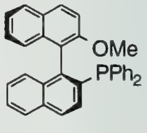
Figure 2

The hydrosilylation of terminal olefins using transition metal catalysts typically favors the formation of the anti-Markovnikov product **17** (equation 4). When 1-octene is hydrosilylated using chloroplatinic acid (H_2PtCl_6) or a triphenylphosphine-palladium complex the 1-trichlorosilyloctane **18** is afforded with high regioselectivity (Scheme 1, entries 1 and 2). When the same olefin is hydrosilylated with Pd(II)-(*S*)-MOP complex, the regioselectivity reverses to afford the 2-silyloctane **17** (entry 3).

Scheme 1

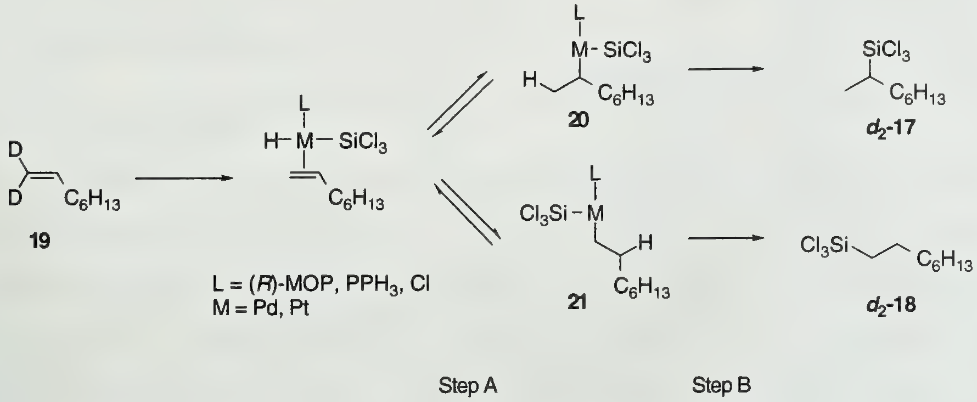


entry	catalyst	temp(°C), time(h)	yield %	ratio (14/15)	ee (%)
1	H_2PtCl_6	25, 2	86	0/100	
2	$[PdCl(\pi\text{-}C_3H_5)]_2\text{-}PPh_3$	40, 24	7	4/96	
3	$[PdCl(\pi\text{-}C_3H_5)]_2$-(*S*)-MOP	40, 24	83	93/7	95



(*S*)-MOP
11

The reaction using the MOP-ligand is proposed proceed via a similar mechanism as suggested for Figure 1. The hydrido(silyl)palladium(II) complex coordinates with the olefin followed by a hydride transfer to either carbon resulting in Pd-alkyl complexes **20** and **21**. Subsequent reductive-elimination to palladium(0) yields **17** and **18**. Hayashi and co-workers conducted a series of deuterium labeling experiments to help rationalize the unusual regiochemistry observed when the MOP ligand is employed.[23] The hydrosilylation reaction of 1,1'-dideutero-1-octene **19** was carried out using the catalysts Pd-MOP, H_2PtCl_6, and Pd-PPh_3. Products and recovered starting material were analyzed for product ratios and location of deuterium. When chloroplatinic acid was used, the 1-silyloctane d_2-**18** was favored in very high regioselectivity and the location of the deuterium was retained in both product and starting material. High regioselectivity favoring the terminal silane d_2-**18** was also observed when Pd-PPh_3 was used but the reverse is seen when Pd-MOP is the catalyst. Scrambling of deuterium is detected in the recovered starting material and products for both palladium complexes. Based on these results, it can be proposed that the regiodetermining step is different for chloroplatinic acid and the palladium complexes. No equilibration occurs between **20** and **21** when H_2PtCl_6 is the catalyst so formation of the intermediate **21** is the regiodetermining step (Step A). Equilibration does occur when either of the palladium catalysts are used as evidenced by the scrambling of deuterium. The regiodetermining step must then be the formation of product (Step B). The reverse in regioselectivity is probably due to the steric environment created by the MOP ligand making formation of the 2-silylalkane d_2-**17** a lower energy pathway when the Pd-MOP catalyst is used.



CONCLUSIONS

Terminal olefins and 1,3-dienes can be hydrosilylated in moderate to good enantioselectivities, when palladium(II) complexes coordinated to a chiral phosphine ligand are used as catalyts,. High yields, catalytic activities, and regioselectivities are also observed in these reactions. The development of the MOP catalyst was a tremendous breakthrough for the hydrosilyation of 1-alkenes, styrenes, and bicyclic olefins. The (*R*)-(*S*)-PPFA-Pd complex worked best with the hydrosilylation of conjugated dienes but only moderate enantiomeric excesses were observed. To fully exploit the utility of this methodology, a clear understanding of the mechanism of the reaction is needed which could open avenues for new chiral ligand design.

While palladium-catalyzed asymmetric hydrosilylation reactions of prochiral olefins have evolved over the last 25 years, the methodology is not completely developed. In light of the recent advances in the synthetic utility of chiral silanes, this class of reactions should be an active area to continue further research. The oxidation of enantioenriched silanes to alcohols with retention of configuration (*vida supra*) is one aspect highlighting the utility of the methodology.[5] Chiral allyl silanes have also been shown to react with electrophiles in asymmetric S_E' reactions.[16] Enantioenriched alkylsilanes have also been useful intermediates in palladium-catalyzed cross-coupling reactions with aryl triflates.[24] Although these organosilanes can be prepared by alternative methods,[25] the procedures are rarely as direct and simple as that of palladium-catalyzed hydrosilylations.

REFERENCES

1. Harrod, J. F.; Chalk, A. J. *J. Am. Chem. Soc.* **1965**, *87*, 16-21.
2. For reviews see: a) Ojima, I.; Nuria, C.; Bastos, C. *Tetrahedron* **1989**, *45*, 6901-6939. b) Horn, K.A. *Chem. Rev.* **1995**, *95*, 1317-1350.
3. Speier, J. L.; Ryan, J. W. *J. Am. Chem. Soc.* **1964**, *86*, 895.
4. Kiso, Y.; Yamamoto, K.; Tamao, K.; Kumada, M. *J. Am. Chem. Soc.* **1972**, *94*, 4373-4374.
5. a) Tamao, K.; Ishida, N.; Tanaka, T.; Kumada, M. *Organometallics*, **1983**, *2*, 1694-1696. b) Tamao, K.; Ishida, N. *J. Organomet. Chem.* **1984**, *269*, C37-C39.
6. a) Chalk, A. J.; Harrod, J. F. *J. Am. Chem. Soc.* **1964**, *86*, 1776-1779. b) Chalk, A. J.; Harrod, J. F. *J. Am. Chem. Soc.*, **1966**, *88*, 3491-3496. c) Sommer, L. H.; Lyons, J. E.; Fujimoto, H. *J. Am. Chem. Soc.*, **1969**, *91*,7051-7061.
7. Marciniec, B.; Mackowska, E.; Gulinski, J.; Urbaniak, W. *Z. Anorg. Allg. Chem.* **1985**, *529*, 222-228.
8. a) Hatanaka, Y.; Goda, K.; Yamashita, F.; Hiyama, T. *Tetrahedron Lett.* **1994**, *35*, 7981-7982. b) Ohmura, H.; Matsuhashi, H.; Tanaka, M.; Kuroboshi, M.; Hiyama, T.; Hatanaka, Y.; Goda, K. *J. Organomet. Chem* **1995**, *499*, 167-171.
9. A private communication with Prof. Tamio Hayashi on the role of hydrosilanes Jan. 31, 1996.
10. Rosini, C.; Franzini, L.; Raffailli, A.; Salvadori, P. *Synthesis* **1992**, 503-517.
11. Uozumi, Y.; Hayashi, T. *J. Am. Chem. Soc.* **1991**, *113*, 9887-9888.
12. a) Hayashi, T. *Pure and Appl. Chem.* **1988**, *60*, 7-12. b) Hayashi, T.; Mise, T.; Fukushima, M.; Dagotani, M.; Nagashima, N.; Hamada, Y.; Matsumoto, A.; Kawakami, S.; Konishi, M.; Yamamoto, K.; Kumada, M. *Bull. Chem. Soc. Jpn,* **1980**, *53*, 1138-1151.
13. a) Marinetti, A.; Ricard, L. *Tetrahedron*, **1993,** *49*, 10291-10304. b) Marinetti, A.; Ricard, L. *Organometallics* **1994**, *13*, 3956-3962. c) Marinetti, A. *Tetrahedron Lett.* **1994**, *35*, 5861-5864.
14. a) Okada, T.; Morimoto, T.; Achiwa, K. *Chem. Lett.* **1990**, *999*. b) Sukaraba, S.; Okada, T.; Morimato, T.; Achiwa, K. *Chem. Pharm. Bull.* **1995**, *43*, 927-934.
15. a) Hayashi, T. *J. Synth. Org. Chem., Jpn.* **1994**, *52*, 28-39. b) Uozumi, Y.; Kitayama, K.; Hayashi, T.; Yanagi, K.; Fukuyo, E. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 713-722. c) Kitayama, K.; Uozumi, Y.; Hayashi. T. *J. Chem. Soc., Chem. Commun.* **1995**, 1533-1534.
16. a) Hayashi, T.; Kabeta, K.; Yamamoto, T.; Tamao, K.; Kumada, M. *Tetrahedron Lett.* **1983**, *24,* 5661-5664. b) Denmark, S. E.; Almstead, N. G. *J. Org. Chem.* **1994**, *59*, 5130-5132. c) Hayashi, T.; Matsumoto, Y.; Ito, Y. *Organometallics*, **1987**, *6*, 884-885.
17. a) Hayashi, T.; Kabeta, K. *Tetrahedron Lett.* **1985**, *26*, 3023-3026. b) Hayashi, T.; Matsumoto, Y. *Tetrahedron: Asymmetry* **1990**, *1*, 151-154.
18. Trost, B. M.; Strege, P. E.; Weber, L.; Fullerton, T. J.; Dietsche, T. J. *J. Am. Chem. Soc.* **1978**, *100*, 3407-3415.
19. Hayashi, T.; Konishi, M.; Kumada, M. *J. Chem. Soc., Chem. Commun.* **1983,** 736.
20. Hayashi, T.; Iwamura, H.; Masaki, N.; Matsumoto, Y.; Uozumi, Y. *J. Am. Chem. Soc.* **1994**, *116*, 775-776.
21. a) Kurz, L.; Lee, G.; Morgans, D.; Waldyke, M. J.; Ward, T. *Tetrahedron Lett.* **1990**, *31*, 6321-6324. b) Uozumi, Y.; Tanahashi, A.; Lee, S.; Hayashi, T. *J. Org. Chem.* **1993**, *58*, 1945-1948. c) Uozumi, Y.; Suzuki, N.; Ogiwara, A.; Hayashi, T. *Tetrahedron* **1994**, *40*, 4293-4302.
22. Uozumi, Y.; Hayashi, T. *Tetrahedron Lett.* **1993**, *34*, 2335-2338.
23. Uozumi, Y.; Kitayama, K.; Hayashi, T. *39th Symposium on Organometallic Chemistry, Japan,* **1992**.
24. a) Hatanaka, Y.; Hiyama, T. *J. Am. Chem. Soc.* **1990**, *112*, 7793-7794. b) Hatanaka, Y.; Goda, K.; Hiyama, T. *Tetrahedron Lett.* **1994**, *35*, 1279-1282. c) Hiyama, T.; Hatanaka, T. *Pure and Appl. Chem.* **1994,** *66*, 1471-1478.
25. a) Zhang, J.; Lou, B.; Guo, G., Dai, L. *J. Org. Chem.* **1991**, *56*, 1670-1672. b) Hayashi, T.; Matsumoto, Y.; Ito, Y. *Tetrahedron: Asymmetry* **1991**, *2*, 601-612. c) Hayashi, T.; Konishi, M.; Ito, H.; Kumada, M. J. *Am. Chem. Soc.* **1982**, *104*, 4962-4963.

ARTIFICIAL ION CHANNELS

Reported by Yue Zou February 29, 1996

INTRODUCTION

Transmembrane ion transport is an essential process in living cells, which is mediated in biological systems by carriers and ion channels.[1] To increase the permeabilities of membranes, carriers bind their selected ions, diffuse through the membrane, and release the ions on the other side; By contrast, channels are transmembrane tunnels or pores through which selected ions can diffuse. Structurally, natural ion channels are complex proteins that consist of four or five homologous subunits, each of which contains multiple transmembrane α-helices. By facing the hydrophilic sides of certain α-helices, the subunits aggregate to form an ion-conducting channel.[2] Functionally, ion channels play an important role in cell regulation, intercellular communication, energy production, as well as electrical signal generation and transmission. Furthermore, they have potential applications as biosensors and other new technologies.[1]

Artificial ion channels have attracted attention because they can provide fundamental information on structure-function relationships of natural ion channels. Synthetic ion channels are typically of a defined size and are consequently more easily studied than naturally occurring ion channel proteins. In general, artificial transmembrane ion channels possess the following features: (i) the overall length should be approximately 40-50Å to span a bilayer membrane, (ii) a hydrophilic core is needed to facilitate ion migration, (iii) the exterior face of the channel should be hydrophobic to favor the interaction with membrane lipids, and (iv) the hydrophilic head-groups are needed to serve as membrane anchors to favor the extended conformation of the channel. In this abstract, recent work on artificial ion channels in the field of biomimetic and supramolecular chemistry will be discussed.

ARTIFICIAL PROTEIN AND PEPTIDE ION CHANNELS

α–Helical Peptide Bundles

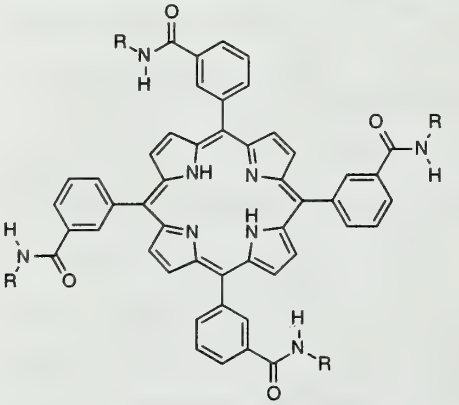
In the 1980s, DeGrado and colleagues designed and synthesized a number of amphiphilic α–helical peptides.[2-7] To mimic natural ion channel proteins, an α–helical peptide approximately 20 amino acids in length is needed to span the biological lipid membrane. Leucine (L, having a nonpolar side chain and high helix-forming propensity) and serine (S, having a polar but uncharged side chain) were chosen as the components for the amphiphilic α–helical peptides.

The circular dichroism spectra of two 21-residue peptides---H_2N-(LSSLLSL)$_3$-$CONH_2$ (**1**) and H_2N-(LSLLLSL)$_3$-$CONH_2$ (**2**) in methanol were typical of α–helical peptides.[2,4] The Mueller-Montal planar bilayer method was used to study their abilities to transport ions in diphytanoyl phosphatidyl choline lipid membranes. Peptide **1** produced square-wave current pulses characteristic of transmembrane channels when switched between conductive and nonconductive states. The time between

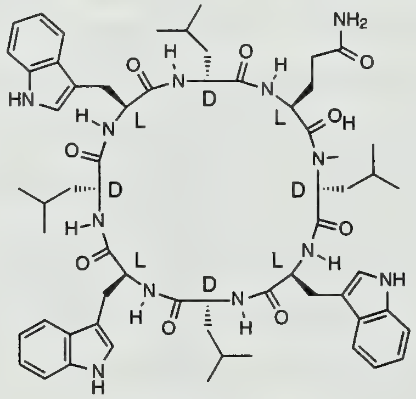
Copyright © 1996 by Yue Zou

channel openings depended on the transmembrane voltage and on the peptide concentration. The cation channels produced by **1** were non-selective for alkali metal ions. Peptide **2** formed proton-selective channels which were less conductive and had a shorter open lifetime. Molecular modeling studies showed that the channel formed by **1** was a hexamer or larger aggregate, whereas that formed by **2** was trimer or tetramer.

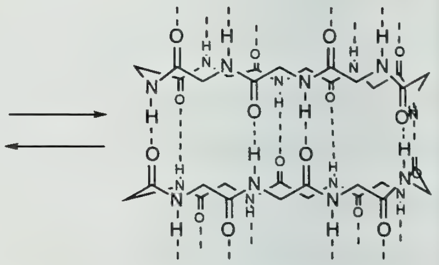
In 1992, DeGrado and co-workers reported work on a four-helix proton selective channel, **3**, built on a tetraphenylporphyrin template.[6] Tetraphilin **3** was prepared by attaching four copies of peptide H_2N-(LSLBLSL)$_3$-CONH$_2$ (B = α-aminoisobutyric acid, introduced to increase the solubility) to *meso*-tetrakis(3-carboxyphenyl)porphyrin via *m*-carboxamido linkages. The attachment of the peptide to the template stabilized the conducting state of the channel and made the formation of channels voltage-independent. This indicated that tetraphilin **3** formed helical bundles that were predominantly vertical in the membrane, even in the absence of a transmembrane voltage.



3 R=-(LSLBLSL)$_3$-CONH$_2$



4



Self-assembling Peptide Nanotubes

In 1994, Ghadiri *et al.* reported a new class of artificial ion channels based on self-assembling cyclic peptides.[8-14] These cyclic peptides (monomers) contain an even number of alternating D- and L-amino acid residues. As a result, the peptides can adopt a flat ring-shaped conformation in which all backbone amide planes lie approximately perpendicular to the plane of the structure. Under favorable conditions, the monomers stack and participate in backbone-backbone intermolecular hydrogen bonding in an antiparallel β-sheet pattern to produce a hollow tubular structure with peptide side chains lying on the outside of the ensemble.[8] This structure is supported by electron microscopy, electron diffraction, Fourier-transform infrared spectroscopy and molecular modeling.[8,14]

An ensemble of eight to ten monomers (40-50 Å) would be long enough to traverse the lipid membrane. When a sufficient concentration of monomer is incorporated in lipid bilayer, the transmembrane channel structures could be formed spontaneously because the self-assembly of the monomers is thermodynamically favored by hydrogen-bonding interactions (enthalpic contribution) and side-chain-lipid hydrophobic interactions (entropic contribution).[9]

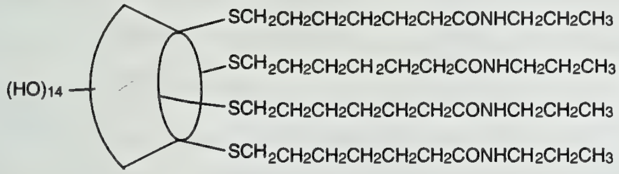
The eight-residue cyclic peptide *cyclo*[-(Trp-D-Leu)$_3$-Gln-D-Leu-] (**4**) was designed as the ion channel-forming monomer and synthesized on solid support.[9,14] When inserted in phosphatidylcholine vesicles, **4** self-assembled to form a 7.5 Å (diameter) channel structure with hydrophobic surface (Trp and Leu residues), which is supported by Fourier-transform infrared spectroscopy. Proton transport from outside to inside the vesicles was studied by measuring the fluorescence intensity of an entrapped pH-sensitive dye. *Cyclo*[-(Trp-D-Leu)$_3$-Gln-D-Leu-] showed similar proton transport ability as gramicidin A, a natural ion channel. Control studies suggested that the hydrophobic surface characteristic and the ability of the peptides to participate in extended hydrogen-bonded stacking interaction were the two most important features in the cyclic peptides that formed ion channels.

ARTIFICIAL NON-PROTEIN ION CHANNELS

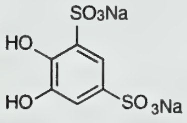
A, C, D, F-Tetrasubstituted *β*-Cyclodextrin

In 1982, Tabushi and co-workers published one of the earliest non-protein channel-forming molecules---A, C, D, F-tetra-6-(6-*n*-butyrylamino-*n*-hexyl-1-sulfenyl)-*β*-cyclodextrin (half-channel, **5**).[15] When incorporated in egg lecithin (EL) vesicles, two molecules of half channel **5** formed an ion channel by aggregating as a tail-to-tail dimer. This channel aligned comfortably along lecithin molecules and bound the metal ion moderately well in one of several equally-spaced binding sites.

The rates of metal ion (Cu^{2+} and Co^{2+}) transport across the membrane incorporated with **5** were measured by detecting the absorption at 310 nm of **6**·M^{2+} complex (**6**, an artificial liposome incorporating Tiron entrapped inside the vesicles). The results indicated that the rate of Co^{2+} and Cu^{2+} transport by **5** were much faster than that of by 18-azacrown-N6, a specific carrier of these metals.


SCH2CH2CH2CH2CH2CH2CONHCH2CH2CH3
SCH2CH2CH2CH2CH2CH2CONHCH2CH2CH3
(HO)14
SCH2CH2CH2CH2CH2CH2CONHCH2CH2CH3
SCH2CH2CH2CH2CH2CH2CONHCH2CH2CH3

5

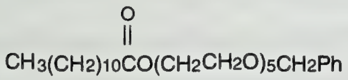


6

Fatty Acid Ester with Polyether Chain

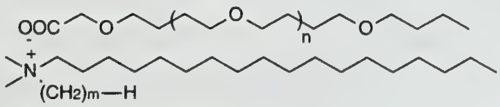
In 1990, a very simple artificial channel-forming molecule **7** was reported by Menger and colleagues.[16] A polyether chain provided the ion-conducting segment which was only sufficiently long enough to span one layer of lipid bilayer. A minimum of two molecules (one in each layer) must align to form a membrane-spanning ion channel. The benzyl group attached to the polyether presumably associates with the head-groups of the lipid by an ion-dipole interaction to serve as membrane anchor. The saturated hydrocarbon tail fosters the stability of **7** in the hydrophobic membrane.

Compound **7** was inserted in the distearoyl phosphatidycholine (DSPC) vesicles, and proton transport was monitored by fluorescense spectroscopy of a pH-sensitive fluorescent dye encapsulated in the vesicles. The results showed that the proton-transporting ability of **7** exceeds that of gramicidin A.



$CH_3(CH_2)_{10}\overset{\overset{O}{\|}}{C}O(CH_2CH_2O)_5CH_2Ph$

7



8

(n=2, 3; m=18)

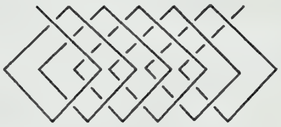
Amphiphilic Carboxylated-ammonium Ion Pairs

Kobuke and co-workers synthesized ion channel-forming molecules based on polyether chain of the amphiphilic salts such as **8**.[17] These molecules are composed of oligoether-glycolates complexed with dialkyldimethylammonium cation. The ability of ion transport was detected by using single-channel measurement in planar bilayer system. The observed conductance levels ($10\text{-}10^3$ pS) were large and demonstrated that **8** was as active as gramicidin A. When incorporated in a lipid bilayer, the molecules self-associated to form half-channel because of the tendency of the hydrophilic polyether chain to separate from the hydrophobic membrane lipid. The head-groups (ion pairs) served as membrane anchors, and the polyether chains constituted the polar inner surface of the channel. The long alkyl chains from ammonium counterparts formed the outer hydrophobic wall that stabilized the polar pore. The connection of two half-channels in different layers of the lipid bilayer formed a cation selective channel. These ion channels showed no selectivity for monovalent cations.

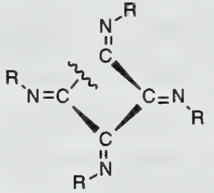
Isocyanide Polymer with Benzo-18-crown-6 Side Chains

In 1984, Nolte *et al.* reported a promising artificial ion channel based on an isocyanide polymer, $(\mathrm{R{-}N{=}C{<}})_n$ (**9**), which contains benzo-18-crown-6 side chains.[18] The rigid helical conformation (with four repeating units per turn) of the isocyanide polymer facilitates the stacking of crown ether rings on the top of each other to form four channels which run parallel to the polymer helical axis.

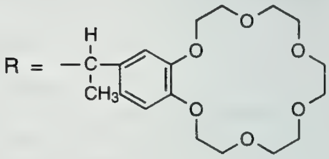
Carbon Backbone Side view



Top View



9



Compound **9** was incorporated in dihexadecyl phosphate (DHP) vesicles, and the rate of cobalt ion transport was measured by following either the decrease in absorption of the entrapped dye mono sodium 4-(2-pyridylazo)resorcinol (PAR) at ~400 nm or the increase in absorption of the cobalt-PAR complex at 510 nm. The studies showed that **9** enhanced the ion permeability of the membrane bilayer of DHP vesicles, and the transport of cobalt ions was coupled to a counter-transport of protons (antiport) in order to conserve electroneutrality.

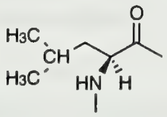
Determination of ion transport rate as a function of temperature afforded an Arrhenius activation energy E_a=24 kJ/mol, which is consistent with an ion channel mechanism (E_a=20.5-22.5 kJ/mol)[19a] rather than a carrier mechanism (E_a=90-120 kJ/mol).[19b]

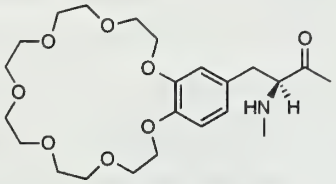
Tubular Hexacrown Molecule

Voyer and Robitaille reported an ion channel based on crown ethers attached to a peptide backbone.[20,21] The designed molecule **10** composed of 15 L-leucine residues and six 21-crown-7 L-phenylalanine residues (**11**) was prepared by solid phase peptide synthesis on an oxime resin. Circular dichroism studies of **10** in trifluoroethanol (TFE) suggested that this 21 amino acid peptide could adopt a stable α-helical conformation in the low-polarity environment such as a bilayer membrane. The six crown ether rings aligned on the same side of the α-helical peptide to form a channel long enough to span the membrane bilayer. The pH-stat method demonstrated that **10** functions as an artificial ion channel in membrane bilayer, and facilitates alkali metal ion transport as efficiently as gramicidin A.

N-t-Boc-(Leu-**11**-Leu-Leu-Leu-**11**-Leu)$_3$-OMe **10**

Leu=L-Leucine=

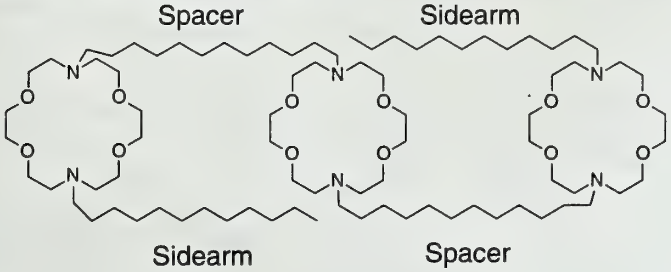




11

Tris(macrocyclic) systems

A series of tris(macrocyclic) compounds were synthesized by Gokel *et al.* as models for cation-conducting channels, and their structure-activity relationships were studied.[22,23] In general, the tris(macrocyclic) systems have the structure "sidearm-crown-spacer-crown-spacer-crown-sidearm".



12

^{23}Na-NMR rate studies showed that when incorporated in egg lecithin vesicles, compound **12** (C_{12}<N18N>C_{12}<N18N>C_{12}<N18N>C_{12}) served as a unimolecular ion channel that enhanced sodium ions permeability. The efficiency of **12** was ca. 100-fold poorer than that of gramicidin at 40 µM concentration. The same rate of Na$^+$ transport was observed for the compound without the two sidearms (H<N18N>C_{12}<N18N>C_{12}<N18N>H). However, if both sidearms of **12** were replaced by benzyl groups ($PhCH_2$<N18N>C_{12}<N18N>C_{12}<N18N>CH_2Ph), the rate of Na$^+$ flux increased by 40%. This suggests that the arene residues probably serve as membrane anchors, which remain outside of the membrane and interact with the polar head-groups of the lipid. This weak interaction further stabilizes the extended conformation of the molecule in the membrane.

The molecule lost its cation-conducting ability if the distal diaza crown ether rings of **12** were replaced by aza-18-crown-6 macrocycles (<18N>C_{12}<N18N>C_{12}<N18>). This observation suggests that protonation of the diaza-18-crown-6 rings may provide an alternative mechanism for membrane anchoring, where the two distal macrocycles serve as polar head-groups to insure the traverse orientation of the molecule in the membrane and provide a cation entry or exit point. The central macrocycle acts as a central cation relay because alteratings affect its ability to conduct cations.

The spacers link the macrocycles and complete the amphiphilic structure that favors the hydrophobic interaction between the molecule and the membrane. Replacement of the nonpolar spacers with polar spacers eliminates the cation channel function of the molecule.

Bouquet-shaped Molecules Based on Macrocycle and β-Cyclodextrin Cores

Lehn and co-workers began their studies on "bouquet-shaped molecules" in 1988.[24-29] Six bouquet-shaped molecules ($\mathbf{B_{M}^{o}}$, $\mathbf{B_{M}^{c}}$, $\mathbf{B_{CD}^{o}}$, $\mathbf{B_{CD}^{c}}$, $\mathbf{C_{M}^{o}}$ and $\mathbf{C_{M}^{c}}$, Figure 1) were designed and synthesized.[24-26] In molecule $\mathbf{B_{M}^{o}}$, two bundles of four poly(oxyethylene) chains with polar carboxylate head-groups (membrane anchors) were grafted on a central 18-crown-6 derivative which served as framework for the bundles and as ion selective site (relay point). Molecule $\mathbf{B_{CD}^{o}}$ possessed two bundles of seven poly(oxyethylene) chains grafted on the more rigid but larger β -cyclodextrin (diameter *ca.* 7.0 Å). $\mathbf{B_{M}^{c}}$ and $\mathbf{B_{CD}^{c}}$ are the polyalkyl analogues of $\mathbf{B_{M}^{o}}$ and $\mathbf{B_{CD}^{o}}$, respectively. The fully extended conformations of these four bouquet molecules (length *ca.* 45-50 Å) are long enough to traverse the membrane bilayer to form unimolecular ion channels. $\mathbf{C_{M}^{o}}$ and $\mathbf{C_{M}^{c}}$ are shorter analogues of $\mathbf{B_{M}^{o}}$ and $\mathbf{B_{M}^{c}}$, respectively.

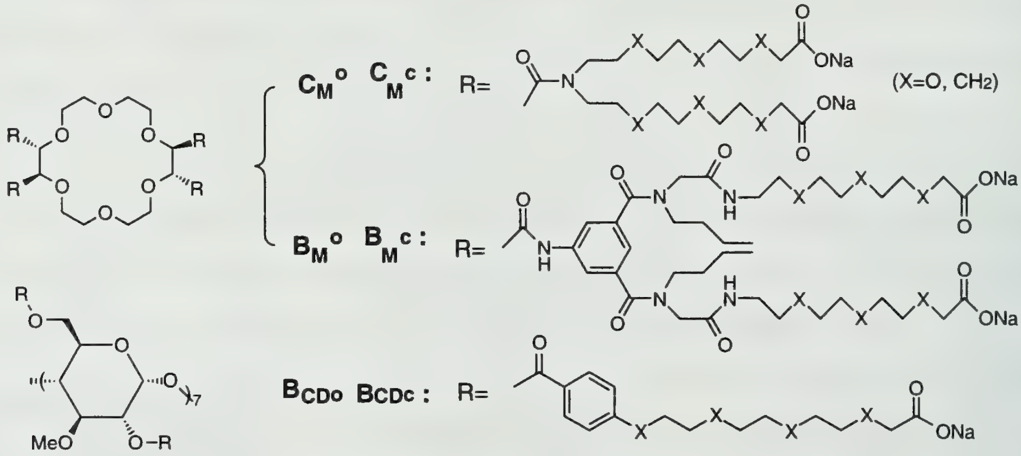


Figure 1. Structures of bouquet-shaped molecules

These bouquet molecules were successfully incorporated in dipalmitoyl phosphatidylcholine (DPPC) or egg phosphatidylcholine (EPC) vesicles.[27,28] Their ability to transport alkali-metal ions through lipid membranes was established by NMR spectroscopy. The influx of Na^+ ions into the vesicles was followed by ^{23}Na NMR spectroscopy, and the efflux of Li^+ ions was followed simultaneously by ^{7}Li NMR spectroscopy. A chemical shift reagent allowed metal ions inside and outside the vesicles to be distinguished.[27,29] The results show that (i) all the bouquet molecules transport ions rather slowly and at similar rates in fluid membranes; (ii) the transport of Na^+ and Li^+ by bouquet molecules is coupled and follow an antiport fashion; (iii) both $\mathbf{B_{M}^{c}}$ and $\mathbf{B_{CD}^{o}}$ transport ions at similar rates in fluid membranes and gel-state membranes, whereas $\mathbf{B_{M}^{o}}$, $\mathbf{B_{CD}^{c}}$, $\mathbf{C_{M}^{o}}$ and $\mathbf{C_{M}^{c}}$ lose their ion transport capabilities in gel-state membranes; (iv) the rate of ion transport by $\mathbf{B_{M}^{c}}$ increases with increasing $\mathbf{B_{M}^{c}}$ concentration, whereas the transport rate by $\mathbf{B_{M}^{o}}$ is concentration independent. The above evidence suggests that $\mathbf{B_{M}^{c}}$ and $\mathbf{B_{CD}^{o}}$ transport ions by the channel mechanism, while $\mathbf{C_{M}^{o}}$ and $\mathbf{C_{M}^{c}}$ operate via a carrier mechanism. Ion transport by $\mathbf{B_{M}^{o}}$ is thought to occur by a process other than channel and carrier mechanism.

Channel-forming Molecules Based on Macrocycle Esters

Fyles *et al.* designed a series of channel-forming molecules based on macrocycle esters to mimic ion channels that are very similar to the bouquet-shaped molecules. The molecules are composed of a polar "core" unit and two to six amphiphilic "wall" units with polar "head" units (Figure 2).[30-34] The core unit, based on polycarboxylate crown ethers derived from tartarate, is designed to lie near the mid-plane of the membrane bilayer. The wall units, based on macrocyclic tetraesters of maleic acid, radiate from the core to provide relay points for ions and can also interact with the lipid. The head units are attached to the end of the wall units to serve as membrane anchors to assist in the transmembrane orientation of the molecule.

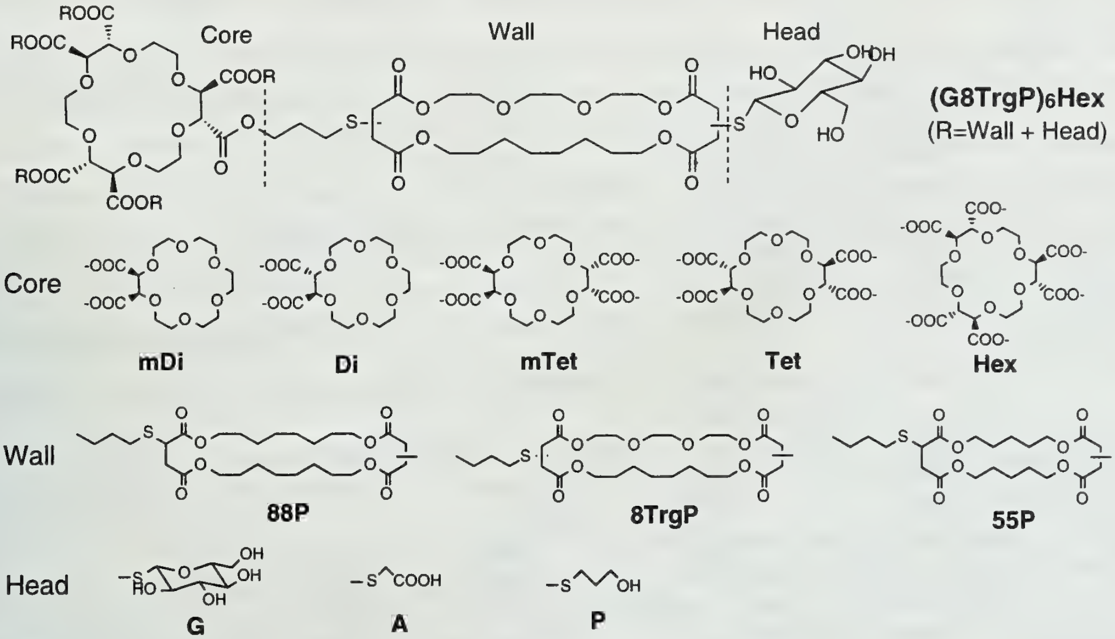


Figure 2. Structures and abbreviations

The ion transport abilities of the molecules were studied by pH-stat techniques in lipid vesicles composed of egg phosphatidyl choline/egg phosphatidic acid/cholesterol in an 8:1:1 molar ratio. The low activation barrier for ion transport and significant inhibition of cation transport by competing cations were proposed as criteria to recognize channel-like transporters. The molecule **(G8TrgP)$_6$Hex** transported ions by a channel mechanism, and was as active as gramicidin. Other channel-forming molecules included **(A88P)$_4$Tet**, **(G88P)$_4$Tet**, **(A8TrgP)$_4$Tet**, **(G8TrgP)$_4$Tet**, **(G55P)$_4$Tet**, and **(G88P)$_6$Hex** (abbreviation shown in Figure 3). Molecules such as **(G88P)$_2$Di**, **(G8TrgP)$_2$Di**, **(G55P)$_2$Di**, **(G8TrgP)$_2$mDi** and **(G8TrgP)$_4$mTet** transported ions by a carrier mechanism. The above evidence suggests that the molecules having four or six wall and head-group units on cores derived from (R,R)-(+)-tartaric acid prefer to form channels in the lipid bilayer. However, fewer wall units or cores based on *meso*-tartaric acid lead to a carrier mechanism for ion transport.

CONCLUSION AND FUTURE WORK

In general, four strategies have been used to construct artificial ion channels: (i) self-associating membrane-spanning monomers that form aggregate channels by mimicking natural ion channel proteins; (ii) half-channel compounds that self-assemble in the membrane to form an ion channel in a manner similar to gramicidin; (iii) macrocyclic agents that stack on the top of each other to form a tube long enough to traverse membrane bilayer; (iv) membrane-spanning bouquet-shaped molecules that form unimolecular ion channels. Overall, many successes have been achieved in the development of artificial cation channels, but construction of anion channels has not been reported. In addition, the mechanism of ion transport in the channel (whether hoping from one binding site to another or fluxing through) is still unknown. Future emphasis should be placed on gated artificial ion channels (channels whose opening can be controlled). Gated channels require dissymmetric derivatives that can respond to membrane potential (voltage-gating), light (photo-gating) or small molecule binding stimulus (receptor-gating).

REFERENCES

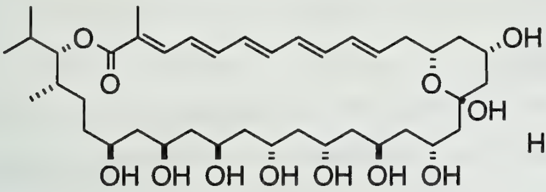
(1) a) Racker, E. *Acc. Chem. Res.* **1979**, 12, 338. b) Voet, D.; Voet, J. G. *Biochemistry* John Wiley & Sons, Inc. **1990**. c) Hille, B. *Ionic Channels of Excitable Membranes* Sinauer: Sunderland 1984
(2) Lear, J. D.; Wasserman, Z. R.; DeGrado, W. F. *Science* **1988**, 240, 1177.
(3) DeGrado, W. F.; Wasserman, Z. R.; Lear, J. D. *Science* **1989**, 243, 622.
(4) DeGrado, W. F.; Lear, J. D. *Biopolymers* **1990**, 29, 205.
(5) Chung, L. A.; Lear, J. D.; DeGrado, W. F. *Biochemistry* **1992**, 31, 6608.
(6) Åkerfeldt, K. S.; Kim, R. M.; Camac, D.; Groves, J. T.; Lear, J. D.; DeGrado, W. F. *J. Am. Chem. Soc.* **1992**, 114, 9656.
(7) Åkerfeldt, K. S.; Lear, J. D.; Wasserman, Z. R.; Chung, L. A.; DeGrado, W. F. *Acc. Chem. Res.* **1993**, 26, 191.
(8) Ghadiri, M. R.; Granja, J. R.; Milligan, R. A.; McRee, D. E.; Khazanovich, N. *Nature* **1993**, 366, 324.
(9) Ghadiri, M. R.; Granja, J. R.; Buehler, L. K. *Nature* **1994**, 369, 301.
(10) Khazanovich, N.; Granja, J. R.; McRee, D. E.; Milligan, R. A.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1994,** 116, 6011.
(11) Granja, J. R.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1994**, 116, 10785.
(12) Engels, M.; Bashford, D.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1995**, 117, 9151.
(13) Clark, T. D.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1995**, 117, 12364.
(14) Hartgerink, J. D.; Granja, J. R.; McRee, D. E.; Milligan, R. A.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1996,** 118, 43.
(15) Tabushi, I.; Kuroda, Y.; Yokota, K. *Tetrahedron Lett.* **1982**, 23, 4601.
(16) Menger, F. M.; Davis, D. S.; Persichetti, R. A.; Lee, J.-J. *J. Am. Chem. Soc.* **1990**, 112, 2451.
(17) Kobuke, Y.; Ueda, K.; Sokabe, M. *J. Am. Chem. Soc.* **1992**, 114, 7618.
(18) a) Neevel, J. G.; Nolte, R. J. M. *Tetrahedron Lett.* **1984**; 25, 2263. b) Kragten, U. F.; Roks, M. F. M.; Nolte, R. J. M. *J. Chem. Soc., Chem. Commun.* **1985**, 1275.
(19) a) Hladky, S. B.; Haydon, D. A. *Biochim. Biophys. Acta.* **1972**, 274, 294. b) Donis, J.; Grandjean, J.; Grosjean, A.; Laszlo, P. *Biochim. Biophys. Acta.* **1981**, 102, 690.
(20) Voyer, N. *J. Am. Chem. Soc.* **1991**, 113, 1818.
(21) Voyer, N.; Robitaille, M. *J. Am. Chem. Soc.* **1995**, 117, 6599.
(22) Nakano, A.; Xie, Q.; Mallen, J. V.; Echegoyen, L.; Gokel, G. W. *J. Am. Chem. Soc.* **1990**, 112, 1287.
(23) Murillo, O.; Watanabe, S.; Nakano, A.; Gokel, G. W. *J. Am. Chem. Soc.* **1995**, 117, 7665.
(24) Jullien, L.; Lehn, J.-M. *Tetrahedron Lett.* **1988**, 29, 3803.
(25) Jullien, L.; Lehn, J.-M. *J. Incl. Phenom. Mol. Recogn.* **1992**, 12, 55.
(26) Canceill, J.; Jullien, L.; Lacombe, L.; Lehn, J.-M. *Helv. Chim. Acta.* **1992**, 75, 791.
(27) Pregel, M. J.; Jullien, L.; Lehn, J.-M. *Angew. Chem. Int. Ed. Engl.* **1992**, 31, 1637.
(28) Jullien, L.; Lazrak, T.; Canceill, J.; Lacombe, L.; Lehn, J.-M. *J. Chem. Soc., Perkin Trans. 2* **1993**, 1011.
(29) Pregel, M. J.; Jullien, L.; Canceill, J.; Lacombe, L.; Lehn, J.-M. *J. Chem. Soc., Perkin Trans. 2* **1995**, 417.
(30) Carmichael, V. E.; Dutton, P. J.; Fyles, T. M.; James, T. D.; Swan, J. A.; Zojaji, M. *J. Am. Chem. Soc.* **1989,** 111, 767.
(31) Fyles, T. M.; James, T. D.; Kaye, K. C. *Can. J. Chem.* **1990**, 68, 976.
(32) Fyles, T. M.; Kaye, K. C.; James, T. D.; Smiley, W. M. *Tetrahedron Lett.* **1990**, 31, 1233.
(33) Cross, G. G.; Fyles, T. M.; James, T. D.; Zojaji, M. *Synlett* **1993**, 449.
(34) Fyles, T. M.; James, T. D.; Kaye, K. C. *J. Am. Chem. Soc.* **1993**, 115, 12315.

SYSTEMATIC STEREOCHEMICAL DETERMINATION OF POLYENE MACROLIDE ANTIBIOTICS: DETERMINATION OF THE STEREOCHEMISTRY OF ROFLAMYCOIN AND FILIPIN III

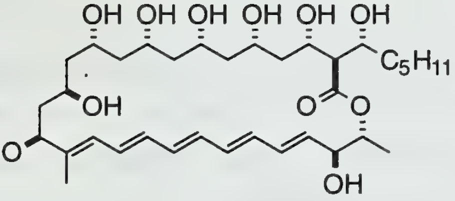
Reported by R. Bruce Diebold March 4, 1996

INTRODUCTION

A systematic procedure to determine the stereochemistry of polyene macrolide antibiotics (PMAs) would be useful, and three such methods have recently been proposed. Mori has suggested a strategy in which the absolute stereochemistry of each alcohol is determined by circular dichroic (CD) spectroscopy.[3] Oishi's approach involves ^{1}H NMR of δ-lactone derivatives to obtain the relative stereochemistry of diol pairs.[5] Rychnovsky has proposed a system in which the relative stereochemistry of diol pairs is established by ^{13}C NMR of acetonide derivatives.[6a] The basics of each method will be briefly presented, and application of Rychnovsky's approach to the stereochemical determination of roflamycoin (**1**) and filipin III (**2**) will be described.



1



2

BACKGROUND

Polyene macrolides are usually produced by soil bacteria of the genus *Streptomyces*. The principal structural feature of PMAs is a 20- to 44- membered lactone ring containing three to eight conjugated olefins and a span of skipped hydroxyls.[1a] A sugar residue, usually mycosamine, is often present as well. The first PMA was discovered in 1950, and over 200 PMAs have since been isolated. The covalent stuctures of about forty have been elucidated,[1a] and the stereochemistry of twelve has been established.

Although most PMAs display potent antifungal activity, few have been found suitable for clinical use.[1b] The principal problem associated with use of PMAs is severe toxicity resulting from a lack of selectivity in vivo. Association of PMAs with the ergosterol present in fungal cell membranes results in the formation of channels through which small metabolites and ions escape. Polyene macrolide antibiotics interact in the same manner with the cholesterol present in mammalian cell membranes, producing similar effects in the host. The PMAs suitable for clinical use are adinistered topically or orally for treatment of the skin, vagina, or gastrointestinal tract. Side effects are relatively minor, but in the case of oral treatment do include diarrhea, nausea, and vomiting. One PMA, amphotericin B, is parenterally administered to treat systemic fungal infections. It produces severe side effects when

Copyright © 1996 by R. Bruce Diebold

utilized in this manner, including shock, renal damage, and cardiac arrhythmia, yet is often the only effective treatment for otherwise fatal infections.

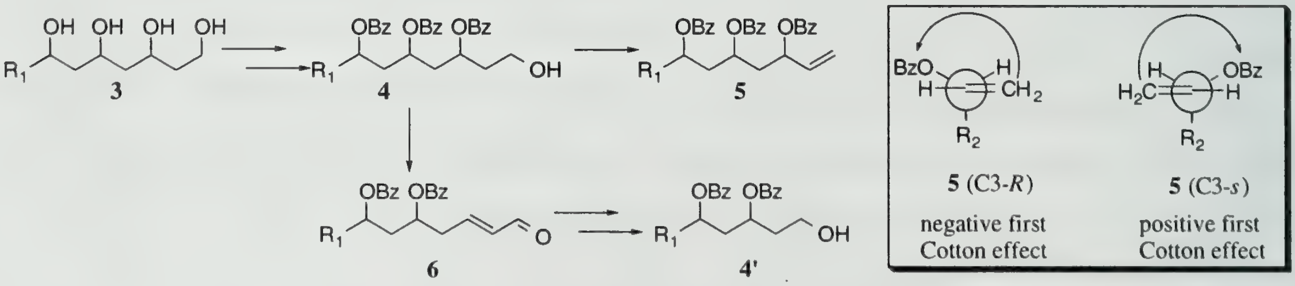
The development of drugs that are more selective for ergosterol over cholesterol would clearly be desirable, but SAR studies have been hindered by the lack of knowledge of the stereochemistry of the skipped polyol systems.[2e] Crystals suitable for X-ray analysis are difficult to obtain, and the structures of only amphotericin B (as the iodoacetonide)[2a] and roxaticin (as the heptaacetate)[2b] have been determined in this manner. More often, NMR spectroscopy (^{1}H, ^{13}C, COSY, and NOE) is used to establish stereochemistry, and in two instances (candidin,[2c] vacidin A[2d]) the stereochemistry of PMAs has been determined by NMR experiments alone. More typically, stereochemical determination of PMAs involves NMR analysis of numerous degradation products to determine as many relative stereochemical relationships as possible. Any unresolved stereochemical relationships are then evaluated by synthesis of the remaining possible diastereomers of the degradation products. The stereochemistry of mycoticins A and B,[2e,f] pimaricin,[2g] nystatin A_1,[2h] lienomycin,[2i] and pentamycin[2j,k] have been examined in this manner.

ITERATIVE DEGRADATION APPROACHES

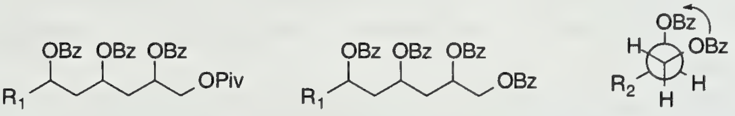
Mori and Oishi have each developed iterative degradation strategies for the stereochemical determination of PMAs. Both approaches have the disadvantage of requiring several synthetic transformations for each iteration.

Mori's method involves CD spectroscopy of linear skipped polyol derivatives (Scheme I).[3] The absolute configuration at the allylic position of perbenzoyl olefin **5** is determined from the exciton chirality interaction between the olefin and the allylic benzoyl group. The CD curve of alcohol **4** must first be subtracted from olefin **5** to compensate for the effects of interactions between the benzoyl chromophores. The negatively chiral C3-*R* configuration would then result in a negative first Cotton effect as per the exciton chirality rule,[4] while the opposite would be true for the C3-*S* configuration. The molecule is then degraded and the next stereogenic center examined. Mori used this method to identify the configurations of eight model polyols.[3a] A similar system was developed to identify the stereochemistry of 1, 2, 4 - polyols (Figure 1).[3b] Subtraction of the CD curve of pivalate **7** from that of perbenzoate **8** yielded the CD interaction between the vicinal benzoates. The rotamer with the

Scheme I



terminal benzoate anti to the chain contributes the most to the CD spectrum. Consequently, the C2-*R* configuration is negatively chiral and results in a negative first Cotton effect, while the opposite is true for the C2-*S* configuration. This method was used to identify the configurations of twelve model polyols.

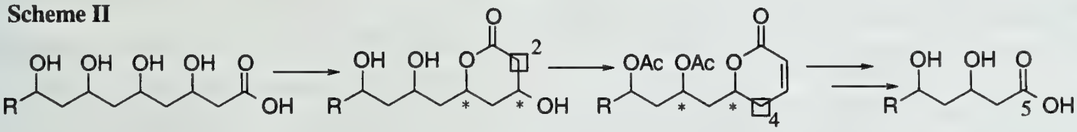


7 **8** **8** (C2-*R*)

Figure 1. Stereochemical determination of C2 of a 1, 2, 4 polyol (Mori's method).

Oishi[5,2j] has proposed a combined iterative degradation / proton NMR strategy (Scheme II). The C2-H_{ax} signal of δ-lactone **10** appears upfield from that of C2-H_{eq} when the substituents at C3 and C5 are syn and downfield when the substituents are anti. Moreover, the signals from the C4 protons of the acetylated α,β unsaturated δ-lactone **11** overlap at 2.35 ppm when the substituents at C5 and C7 are anti, and are distinct when the substituents are syn. These trends were consistently observed in model compounds.[2j] The molecule is then degraded and the analysis reiterated for the next stereogenic alcohol center. Oishi used this procedure to establish the relative stereochemistry of three stereogenic centers in pentamycin.[2j,k]

Scheme II



9 **10** **11** **9'**

^{13}C NMR ANALYSIS OF 1,3-DIOL ACETONIDES

The acetonides of syn and anti 1,3-diols may be distinguished by the chemical shift of the acetonide methyl carbons (Figure 2).[6] Acetonides of syn diols adopt a chair conformation in which the axial methyl carbon (~19 ppm) is readily distinguished from the equatorial carbon (~30 ppm). Acetonides of anti diols take on a twist boat conformation with local C2 symmetry where the methyl carbons are equivalent. As a result, both methyl carbons appear near 25 ppm. The twist boat conformation is preferred due to diaxial interactions in the chair conformation.

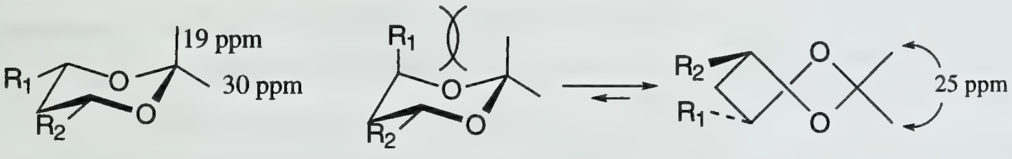


Figure 2. Conformations of syn and anti 1, 3 diol acetonides.

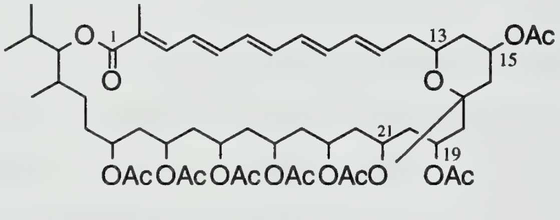
Rychnovsky conducted a complete literature search of 1,3-diol acetonides and found 221 compounds for which the NMR signals of the methyl carbons had been identified.[6c] The shifts of the methyl carbons of 145 syn acetonides occurred at 19.66 ± 0.35 ppm (mean ± SD) and 30.00 ± 0.30, with a mean difference in shifts of 10.34 ± 0.30 ppm. In no instance was the difference in shifts less than 9 ppm. Excluding acetonides bearing nitrile substituents, the differences in shift of the carbons of anti acetonides were at most 4.5 ppm.

Rychnovsky suggested that ^{13}C NMR analysis could be used to identify the number of syn and anti diol acetonides present in a given PMA derivative. In theory, the complete relative stereochemistry of the polyol segment could be determined from just two acetonide derivatives that are frame-shifted with respect to one another.[6a]

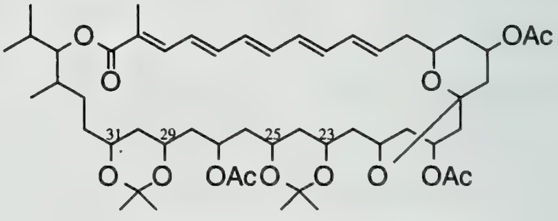
STEREOCHEMICAL DETERMINATION OF ROFLAMYCOIN

In 1971 Schlegel and Thrum isolated roflamycoin from a strain of *Streptomyces roseoflavus*.[7a] An all-trans oxopentaene segment was identified by UV and IR spectroscopy of degradation products.[7b] The remainder of the covalent structure was reported in 1981, based on the EIMS of two permethylated derivatives.[7c]

Rychnovsky reported the complete stereochemistry of roflamycoin in 1994.[8] The relative stereochemistry of C13/C15 and C19/C21 was determined from the peracetylated spriroketal **12** and the diacetonide triacetate spiroketal **13**. The axial protons at C14 and C18 of spiroketal **12** appeared as quartets in the ^{1}H NMR spectrum (*J*= 11.6 and 12.2 Hz, respectively), indicating that the protons at C13, C15, C19, and C21 were all axial. Consequently, the C13/C15 and C19/C21 stereochemical relationships must be anti. Further elaboration of stereochemistry from spiroketal **12** was not possible due to ambiguity in proton assignments.

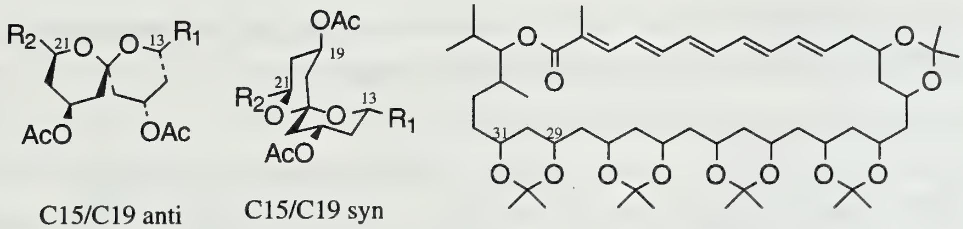


12



13

Near-complete assignment of the proton spectrum of spiroketal **13** allowed determination of the C15/C19 stereochemical relationship. A C15/C19 anti relationship would result in local C2 symmetry of the spiroketal moiety, while a syn relationship would possess in no such symmetry (Figure 3). A large difference between the chemical shifts of the C13 (3.40 ppm) and C21 (4.32 ppm) protons was taken to indicate an absence of symmetry. The substituents at C15 and C19 were concluded to be syn.



C15/C19 anti

C15/C19 syn

14

Figure 3. Possible C15/C19 relationships in spiroketal **13**; pentaacetonide **14**.

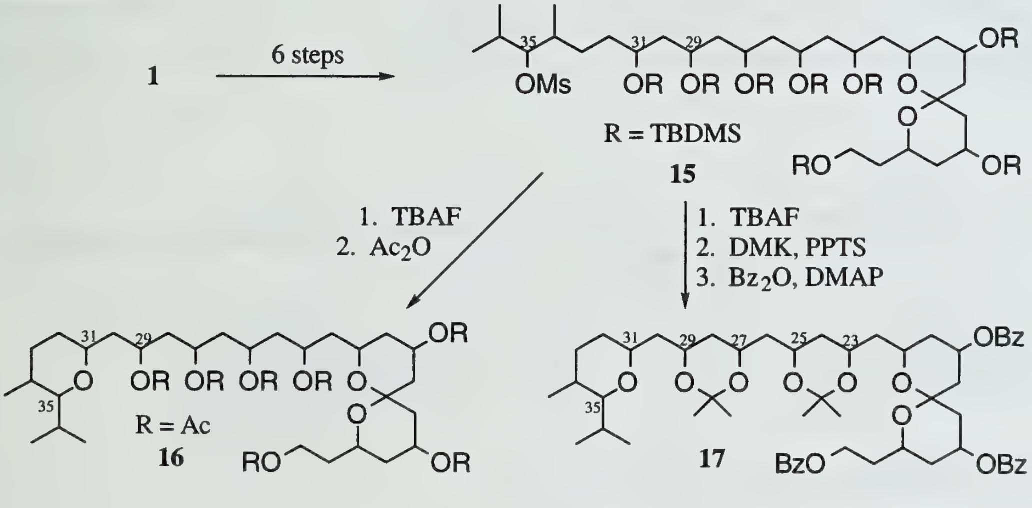
Spiroketal **13** was also used to examine the C23/C25 and C29/C31 relationships. The ^{13}C NMR analysis of spiroketal **13** made from ^{13}C enriched acetone showed signals only at 30.65, 30.48, 19.54, and 19.47 ppm. This indicated that the C23/C25 and C29/C31 relationships were each syn.

The pentaacetonide derivative **14** revealed additional stereochemical detail. Reduction of the roflamycoin hemiketal resulted in a 5:3 mixture of epimers at C17, which, after separation, allowed for preparation of the pentaacetonide. The ^{13}C NMR spectrum of **14** indicated the presence of one syn and four anti acetonides. Since the C29/C31 stereochemical relationship had already been established as syn, the relationships of C27/C25 and C23/C21 had to be anti. In this manner the relative configurations of the entire skipped polyol system was established except for the relationship between C27 and C29 and the configurations of C34 and C35.

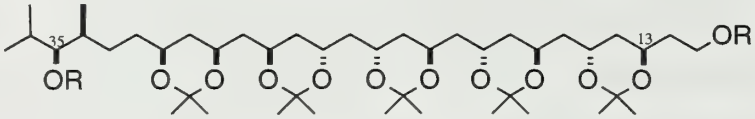
The configurations of C34 and C35 relative to C31 were determined by molecular modeling. The values of $J_{C34\text{-}C35}$ and $J_{C35\text{-}C36}$ of heptaacetylated tetrahydropyran **16** were compared with values generated by Macromodel 3.5 for each of the four diastereomers of (6*R*)-6-ethyl-2-isopropyl-3-methyltetrahydropyran. The results indicated that the configurations at C34 and C35 were syn relative to C31.

The ^{13}C NMR spectrum of diacetonide **17** indicated that the substituents at C27 and C29 were syn. Determination of the relative stereochemistry of roflamycoin was thereby completed, and was subsequently verified by total synthesis of a degradation product.

Scheme III



The absolute stereochemistry of roflamycoin was determined by ^{1}H NMR analysis of the Mosher esters of alcohol **18**. The configuration at C35 was found to be *S*.



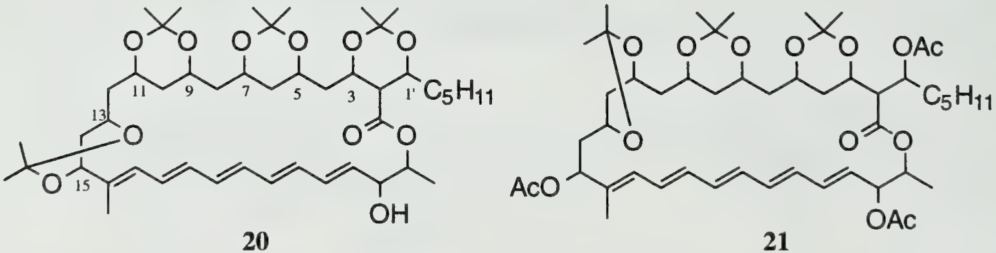
18 R = H
19R R = (*R*)-MTPA
19S R = (*S*)-MTPA

STEREOCHEMICAL DETERMINATION OF FILIPIN III

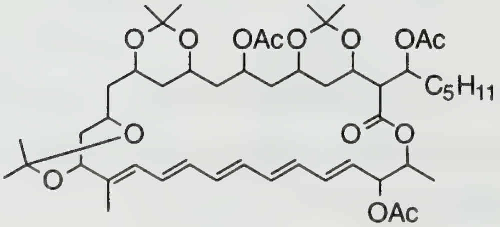
Filipin III is the primary component of an antibiotic mixture isolated from *Steptomyces filipinensis* by Whitfield et. al. in 1955.[1a] This compound is more destructive to organisms with cholesterol-bearing cell membranes than to those bearing ergosterol.[1b]

In 1995, Rychnovsky reported the stereochemistry of filipin III[9] Reaction of the filipin complex with ^{13}C-enriched acetone resulted in formation of one tetraacetonide (**20**) and two triacetonides. The tetraacetonide was separated from the triacetonides by silica gel chromatography, and the triacetonides were then acetylated and separated to yield diacetates **21** and **22**. The ^{13}C NMR of triacetonide **21** clearly showed that all three acetonides were of syn diols, whereas the ^{13}C spectra of acetonides **20** and **22** were ambiguous. The spectrum of each of these compounds indicated the presence of an acetonide with methyl carbon chemical shifts of about 25 and 30-31 ppm.

Literature precedent for the ambiguous acetonide signals can be found in 2,2,4,4,6-pentamethyl-1,3-dioxane.[8] This compound is known to adopt a chair conformation.[10] The axial and equatorial acetonide carbons have chemical shifts of 24.93 and 32.04 ppm respectively. Rychnovsky hypothesized that the ambiguous methyl carbon signals of **20** and **22** were due to an anti acetonide somehow forced into a chair conformation by the rigidity of the cyclic polyene. He also suggested that the chemical shifts of the vinylic acetonides were most likely to be anomalous.



20 **21**

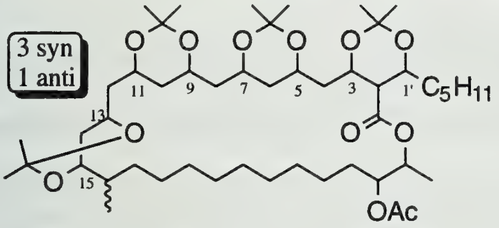


22

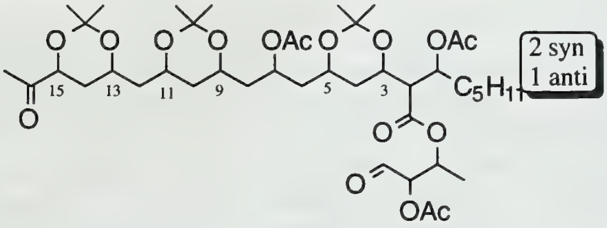
Acetonide	Shift of Methyl Carbons (ppm)
20	31.00, 30.65, 30.50, 30.13, 24.82, 19.97, 19.54, 19.54
21	30.76, 30.70, 30.02, 19.93, 19.82, 19.41
22	30.46, 30.15, 30.05, 24.74, 19.03, 18.97

Figure 4. Acetonide derivatives of Filipin III.

This hypothesis was tested by relieving the rigidity of acetonides **20** and **22**. After protection of the C26 hydroxyl, the olefins of tetraacetonide **20** were hydrogenated to yield the saturated macrocycle **23** as a mixture of epimers at C16. The ^{13}C NMR spectrum of this mixture showed unambiguous methyl signals indicative of three syn and one anti acetonide. Ozonolysis of triacetonide **22** resulted in **24**. The ^{13}C NMR analysis of this compound showed unambiguous methyl signals indicative of two syn and one anti acetonide. Rychnovsky suggested that the relatively large difference in the chemical shifts ($\Delta\delta$ = 1.62) of the methyl carbons of the anti acetonide indicated that the signals came from the acyl substitued acetonide. However, Rychnovsky's review of the ^{13}C NMR spectra of anti acetonides included only two carbonyl-substituted compounds, with $\Delta\delta$ values of 2.6 and 3.6. While it is clear that syn and anti assignments of acetonides may be made on the basis of ^{13}C NMR, conclusions regarding the substituents of acetonides may be premature.



23



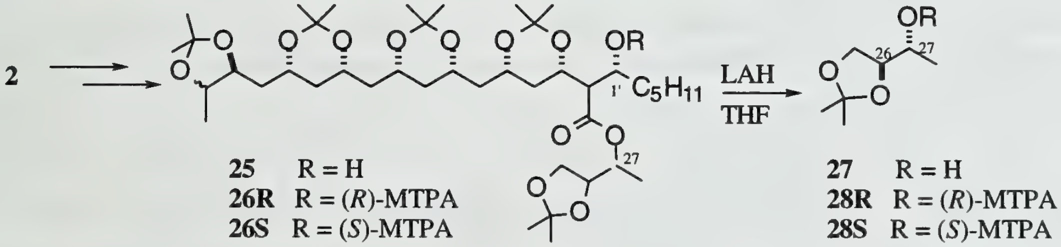
24

The ^{13}C NMR analysis of triacetonide **21** indicates a syn relationship for C3/C5, C7/C9, and C11/C13. The C3/C5 diol acetonide also occurs in triacetonide **24**. Consequently, either the C9/C11 or the C13/C15 acetonide must be anti. The same must be true for the C9/C11 and C13/C15 acetonides in tetraacetonide **23,** defining the C5/C7 and C1'/C3 acetonides as syn. The relatively large $\Delta\delta$ of the anti acetonide of **24** suggests that it is the C13/C15 relationship which is anti.

The C2 proton of tetraacetonide **20** appears in the ^{1}H NMR spectrum as a triplet (J=10.1 Hz). The coupling constant is indicative of diaxial interaction. The substituent at C2 should therefore be anti to the hydroxyls at C3 and C1'.

The absolute stereochemistry of the polyol segment was determined by ^{1}H NMR of the Mosher esters of alcohol **25**, prepared in four steps from filipin. The configuration at C1' was found to be *R*.

Scheme IV



25 R = H
26R R = (*R*)-MTPA
26S R = (*S*)-MTPA

27 R = H
28R R = (*R*)-MTPA
28S R = (*S*)-MTPA

The C26/C27 relative stereochemistry was determined to be anti by direct comparison (^{1}H NMR, GC) of the butane triol derivative **27** with an authentic sample. The absolute stereochemistry at C27 was determined to be *R* by analysis of the corresponding Mosher esters.

Rychnovsky has recently confirmed the stereochemistry of the C1'-C15 segment by total synthesis of a degradation product.[9b]

CONCLUSION

Several systematic methods to determine the stereochemistry of the skipped polyol systems of PMAs have been developed. The iterative degradation methods of Oishi and Mori are useful but require many chemical steps. Rychnovsky's method is the least structured but perhaps the most practical, as demonstrated in the stereochemical determinations of roflamycoin and filipin III.

REFERENCES

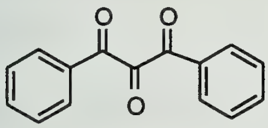
1. (a) Omura, S.; Tanaka, H. In *Macrolide Antibiotics. Chemistry, Biology, and Practice*; Omura, S., Ed.; Academic Press, New York, 1984, pp 351-404. (b) Schaffner, C. P. In *Macrolide Antibiotics. Chemistry, Biology, and Practice*; Omura, S., Ed.; Academic Press, New York, 1984, pp 457-507.
2. (a) Mechlinski, W.; Schaffner, C. P.; Ganis, P.; Avitabile, G. *Tetrahedron Lett.* **1970**, 3873. (b) Maehr, H.; Yang, R.; Hong, L.; Liu, C.; Hatada, M.H.; Todaro, L. J. *J. Org. Chem.* **1989**, *54,* 3816. (c) Pawlak, J.; Sowinski, P.; Borowski, E.; Gariboldi, P. *J. Antibiotics* **1993**, *46*, 1598. (d) Sowinski, P.; Gariboldi, P.; Czerwinski, A.; Borowski, E. *J. Antibiotics* **1989**, *42*, 1631. (e) Schreiber, S. L.; Goulet, M. T. *Tetrahedron Lett.* **1987**, *28*, 6001. (f) Schreiber, S. L.; Goulet, M. T. *J. Am. Chem. Soc.* **1987**, *109*, 8120. (g) Lancelin, J. M.; Beau, J. M. *J. Am. Chem. Soc.* **1990**, *112*, 4060. (h) Lancelin, J. M.; Beau, J. M. *Tetrahedron Lett.* **1989**, *30*, 4521. (i) Pawlak, J.; Nakanishi, K.; Iwashita, T.; Borowski, E. *J. Org. Chem.* **1987**, *52*, 2896. (j) Oishi, T. *Pure & Appl. Chem.* **1989**, *61*, 427. (k) Nakata, T.; Hata, N,; Suenaga, T.; Oishi, T. *Symposium Papers*, 30th Symposium on the Chemistry of Natural Products, Fukuoka, Japan, 1988;Abstract 70.
3. (a) Mori, Y.; Kohchi, Y.; Suzuki, M.; Furukawa, H. *J. Am. Chem. Soc.* **1992**, *114*, 3557. (b) Mori, Y.; Furukawa, H. *Tetrahedron* **1995**, *51*, 6725.
4. (a) Lambert, J. B.; Shurvell, H. F.; Lightner, D.; Cooks, R. E. *Introduction to Organic Spectroscopy,* Macmillan Publishing Company, New York, 1987. (b) Harada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy. Exciton Coupling in Organic Stereochemistry*; University Science Books; Mill Valley, CA, 1983.
5. (a) Nakata, T.; Hata, N.; Nakashima, K.; Oishi, T. *Chem. Pharm. Bull.* **1987**, *35,* 4355. (b) Nakata, T.; Hata, N.; Oishi, T. *Tetrahedron Lett.* **1990**, *31*, 381.
6. (a) Rychnovsky, S. D.; Skalitzky, D. J. *Tetrahedron Lett.* **1990**, *31*, 945. (b) Evans, D. A.; Rieger, D. L.; Gage, J. R. *Tetrahedron Lett.* **1990**, *31*, 7099. (c) Rychnovsky, S. D.; Rogers, B.; Yang, G. *J. Org. Chem.* **1993**, *58*, 3511. (d) Rychnovsky, S. D.; Yang, G.; Powers, J. P. *J. Org. Chem.* **1993**, *58*, 5251.
7. (a) Schlegel, R.; Thrum, H. *J. Antibiotics* **1971**, *24*, 360. (b) Schlegel, R.; Thrum, H. *J. Antibiotics* **1971**, *24*, 368. (c) Schlegel, R.; Thrum, H.; Zielinski, J.; Borowski, E. *J. Antibiotics* **1981**, *34*, 122.
8. (a) Rychnovsky, S. D.; Griesgraber, G.; Schlegel, R. *J. Am. Chem. Soc.* **1994**, *116*, 2623. (b) Rychnovsky, S. D.; Griesgraber, G.; Schlegel, R. *J. Am. Chem. Soc.* **1995**, *117*, 197.
9. (a) Rychnovsky, S. D.; Richardson, T. I. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1227. (b) Rychnovsky, S. D.; Richardson, T. I., submitted for publication in *J. Org. Chem.*
(10) Pihlaja, K.; Kivimaki, M.; Myllyniemi, A. M.; Nurmi, T. *J. Org. Chem.* **1982**, *47*, 4688.

PREPARATION AND SYNTHETIC UTILITY OF VICINAL TRICARBONYL COMPOUNDS

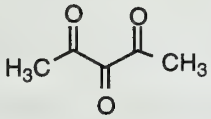
Reported by Qingwu Alan Jin March 7, 1996

Introduction

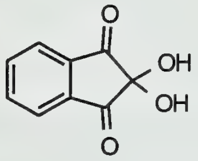
The first reported synthesis of a vicinal tricarbonyl compound (**1**) was published in 1890 by de Neufville and von Pechmann.[1a] Since then, the synthesis of vicinal tricarbonyl compounds has been an active area of reseach, and various methods leading to their construction have been reported.[2] In 1901, Sachs and Barschall[1b] reported the first synthesis of the aliphatic parent tricarbonyl compound, dimethyl triketone, **2**. Ninhydrin (**3**) is probably the best known triketone, and the chemistry of ninhydrin was reviewed by McCaldin in 1960.[1c] Recently, interest in vicinal tricarbonyl compounds has increased dramatically because this subunit has been found in the binding regions of immunosuppressants FK 506[3] and rapamycin[4]. Additionally, this functionality can be used in the synthesis of a wide variety of heterocyclic compounds.



1, 1890, de Neufville
von Pechmann



2, 1901, Sachs; Barschall



3, Ninhydrin
Review: McCaldin, 1960.

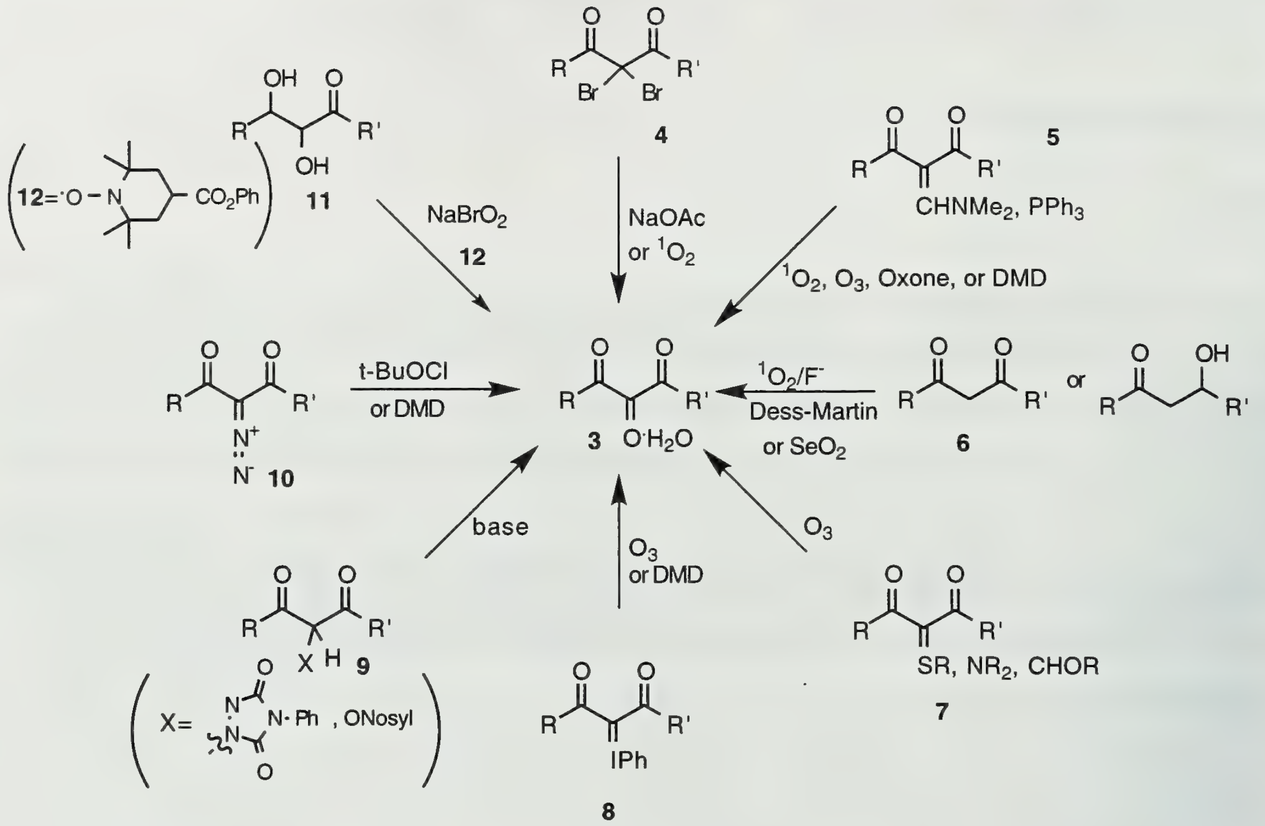
Preparation of tricarbonyl compounds

The chemistry of polyketones prior to 1973 has been reviewed by Rubin. Historically, the syntheses of tricarbonyl compounds include oxidation of β-diketones, oxidation of unsaturated ketones, and rearrangements of tetraketones.[2] More recently, some efficient and selective preparations of tricarbonyl compounds have appeared. The difficulty associated with the preparation of tricarbonyl compounds is their reactivity as they are unstable in basic and acidic condition, and in many cases further reaction of the product is observed. In most cases, hydration of the central carbonyl unit occurs spontaneously during the reaction or workup. The tricarbonyls discussed in this review actually refer to synthetic tricarbonyl equivalents.

A variety of methods have been developed to prepare 1,2,3-triketones, 2,3-diketo esters, and in some

Copyright © 1996 by Qingwu Alan Jin

Scheme I



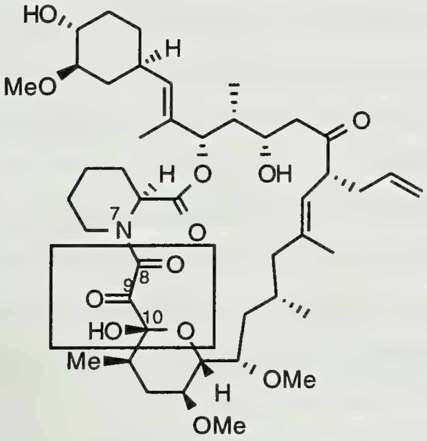
R, R' varies with methods, and can be alkyl, aryl ,O-alkyl, O-aryl ,or N-alkyl groups.

cases 2,3-diketo amides. These methods include (Scheme I, from top, clockwise), (a) dibromination of the central methylene of β-diketone to form compound **4**, followed by oxidative cleavage or hydrolysis of the C-Br bond,[7] (b) oxidative cleavage of C=C or C=P double bond of **5**,[27,12] (c) oxidation of β-diketones or β-hydroxy ketone **6** by various reagents,[5,6] (d) oxidative cleavage of C=S, C=N or enol ether double bond of **7**,[28] (e) oxidation of phenyl iodonium ylides **8** by ozone or dimethyldioxirane (DMD),[29] (f) oxidative functionalization of central methylene of β-diketone to compound **9** followed by hydrolysis,[8,9] (g) oxidation of 2-diazo-1,3-dioxo derivatives **10**[30] and (h) oxidation of 2,3-dihydroxy esters **11**.[10] While these methods differ greatly in efficiency and R, R' groups that can be accommodated in the tricarbonyl unit, DMD cleavage of ylide **8** is probably the most efficient and selective oxidative method.[12c] Many oxidizable functional groups including halides, alkenes (conjugated, or isolated), enol ethers, furan and thiophenes survive these mild conditions.

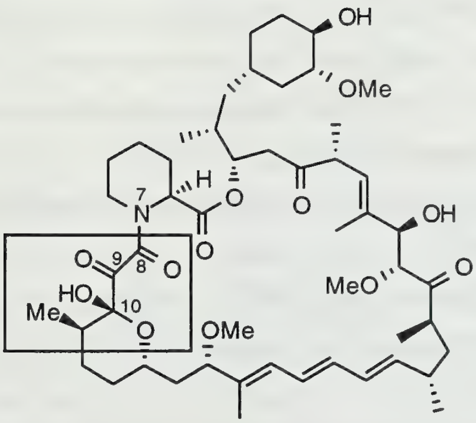
Synthesis of the "tricarbonyl region" of FK-506 and Rapamycin

FK-506 (**13**) and rapamycin (**14**, Scheme II) have recently emerged as potent immunosuppressive agents. Both of these natural products possess a masked α,β-diketo amide system which is believed to play an important role in the binding and T-cell deactivating activities of these molecules.[3b, 4b]

Scheme II



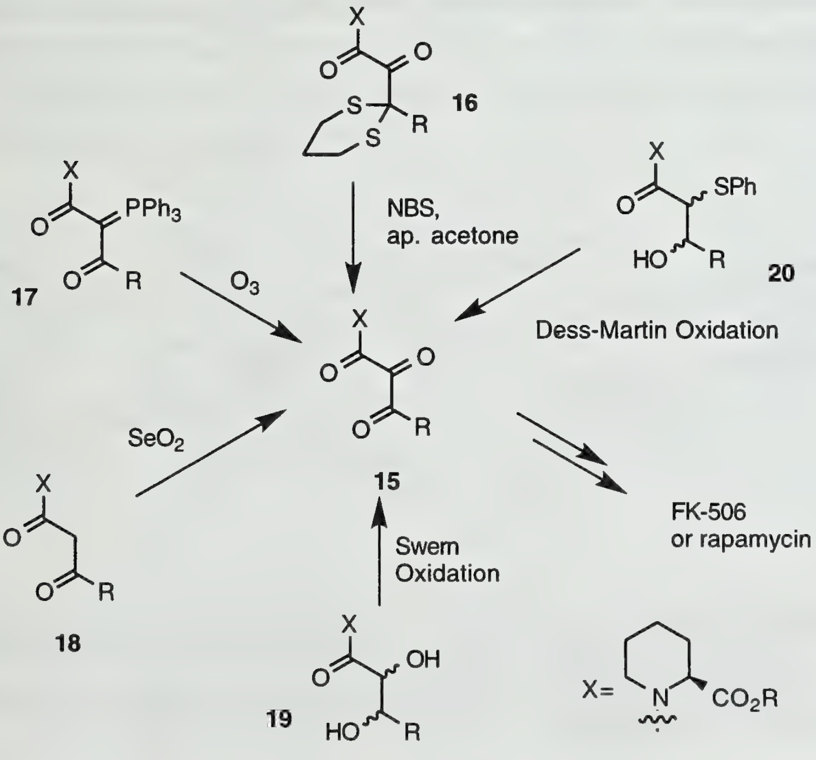
13, FK-506



14, Rapamycin

Total syntheses of FK-506 were reported by the Jones[13] and Schreiber[14] groups in 1989 and 1990 respectively. Although the formal syntheses were achieved, due to the interesting biological properties of these molecules, improvements in the syntheses are still of great importance. In both cases, the tricarbonyl region was generated by an aldol addition of α-hydroxy amide or ester to an aldehyde at C_{10}. This pathway requires selective deprotection of the hydroxyl groups prior to oxidation to the carbonyl group (Scheme III).

Scheme III



Many reports on the synthesis of the tricarbonyl component in FK-506 have appeared. Williams' synthesis[16a] of the tricarbonyl begins with coupling of β-keto ester and acyl chloride. Decarboxylation and oxidation with SeO_2 afforded α,β-diketo amide **15**. In Wasserman's synthesis, keto ylide **17** was synthesized by coupling of an acid chloride with an acyl phosphoranylidene in the presence of bis(trimethylsilyl) acetamide. Oxidative cleavage of **17** using either ozone or singlet oxygen afforded the tricarbonyl compound **15** in high yield.[16b]

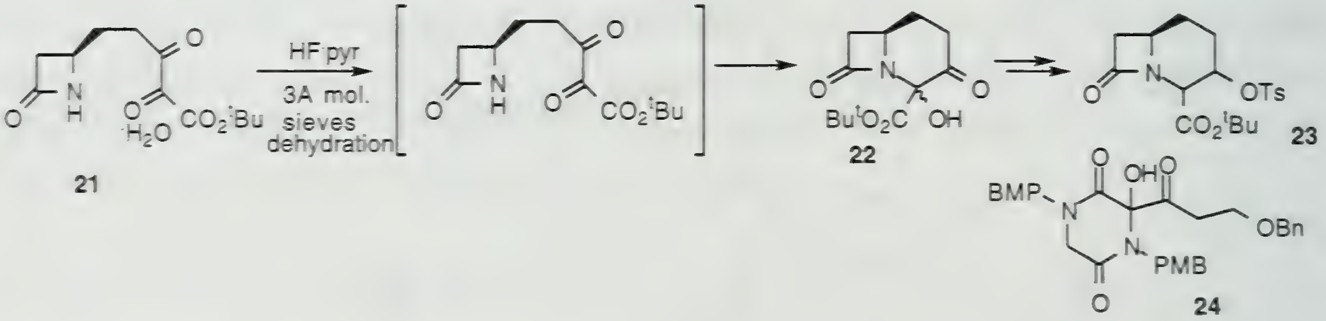
Danishefsky reported two different methods for the synthesis of the tricarbonyl region of FK-506. The first approach coupled dithiane and an ester to afford compound **16**, which upon cleavage of the dithiane gave the tricarbonyl unit.[17a] In the second, coupling of an α(-phenylthio) ketone and an aldehyde afforded β-hydroxy-α(-phenylthio) amide **20**, which was oxidized by Dess-Martin's reagent to the α,β-diketo amide **15**.[17b]

In 1995, Nicolaou, *et. al.*,[15] reported on the total synthesis of rapamycin where two of the three carbonyls were initially constructed as the vicinal diols. The α,β-dihydroxy amide **19** was oxidized to the tricarbonyl by double Swern oxidation prior to the macrocyclic ring closure.

Synthetic application of tricarbonyl compounds

The central carbonyl group of the tricarbonyl molecules exists predominantly as the hydrate. The hydrated and carbonyl forms are believed to be in equilibrium in solution, although the former heavily favored. In the presence of a nucleophile, with or sometimes without a dehydrating reagent, nucleophilic addition to the central carbonyl group can occur. To a large extent, addition at the central carbonyl reduces the unfavorable electrostatic interaction between the carbonyls, and this reactivity can be utilized to promote difficult electrophilic bond forming reactions. Dehydrating reagents are needed in the case of weak nucleophiles such as an amide.

Scheme IV

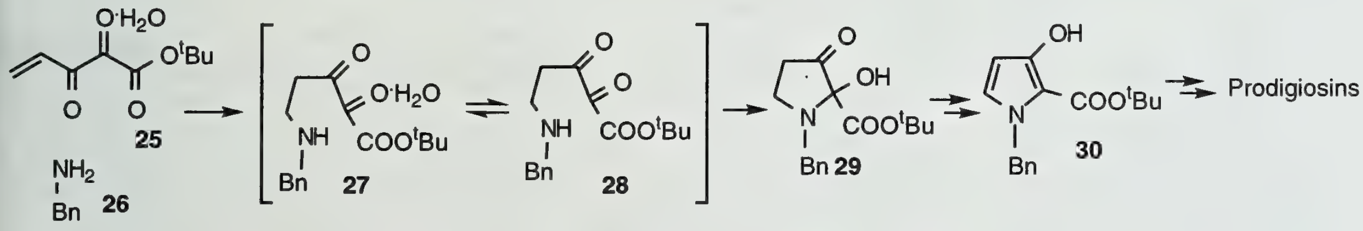


Without the presence of dehydrating reagents, nucleophilic addition is believed to occur to the carbonyl form whose continuous consumption drives the reaction to complete. Attachment of other acceptor sites to the tricarbonyl unit provides opportunities for ring forming reactions with di- and tri-nucleophiles. This methodology has been used in the preparation of di- and tricyclic lactams, indolizidines, pyrroles, and pyrrolidines, and in the synthesis of various natural products.

Because of its high reactivity, the central carbonyl can accept very weak nucleophiles such as amides. Wasserman[18] utilized this unusual reactivity in the synthesis of fused ring β-lactam **23** by nucleophilic addition of the amide nitrogen at the central carbonyl of the tricarbonyl unit. Diketopiperazine **24** was also synthesized by this methodology. Nucleophilic addition of *p*-methoxybenzyl-substituted amide to the central carbonyl occurred with heating to afford compound **24**, which was a key intermediate in the synthesis of bicyclomycin[19] (Scheme IV).

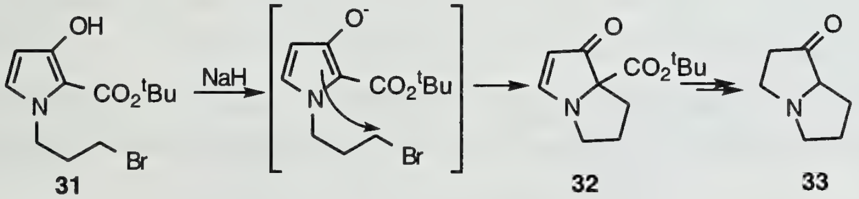
Unsaturated vicinal tricarbonyl compound **25** can serve as a multiple electrophile through nucleophilic conjugate addition at the terminal carbon and 1,2-addition at the central carbonyl group. Reaction of **26** with **25** initially formed amine **27**, and addition at the central carbonyl group afforded pyrrolidine **29**. Compound **29** could be converted to the corresponding 2-carbalkoxy-3-hydroxypyrrole **30** in the presence of silica gel (Scheme V).[20] Compound **30** was used in the synthesis of prodigiosins, which show potent antibacterial, and antifungal activity.

Scheme V



The 3-hydroxypyrrole **31**, which was made in this pathway, could serve as a nucleophile, and intramolecular alkylation afforded the indolizidine **33** (Scheme VI).[23] By varying the length of the alkyl chain, various bicyclic tertiary amines could be prepared. It is noteworthy that pyrrolidine **27** (scheme V) can serve as an electrophile or a nucleophile.

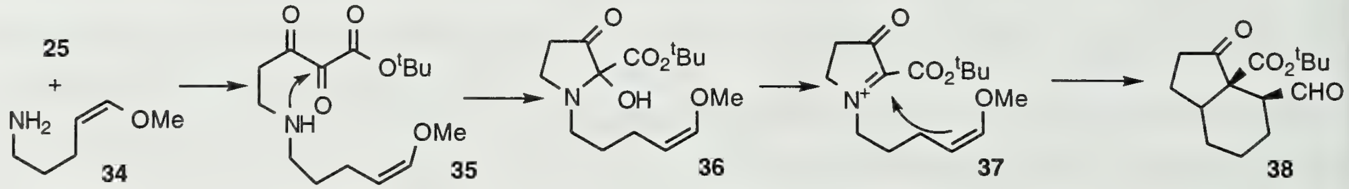
Scheme VI



In a different approach towards functionalized indolizidines, the vinyl tricarbonyl reagent **25** was used as a trielectrophile. The primary amino group initially takes part in a Michael addition to the α,β-unsaturated ketone, and to the central carbonyl after dehydration, to give a hydroxypyrrolidone carboxylate

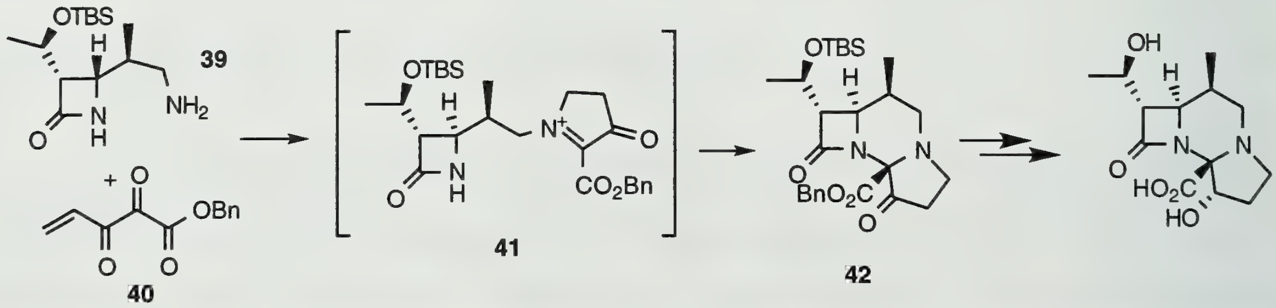
36. Under acidic conditions, **36** dehydrates to form iminium salt **37**, which acts as a third electrophile to form compound **38** (Scheme VII).[21]

Scheme VII



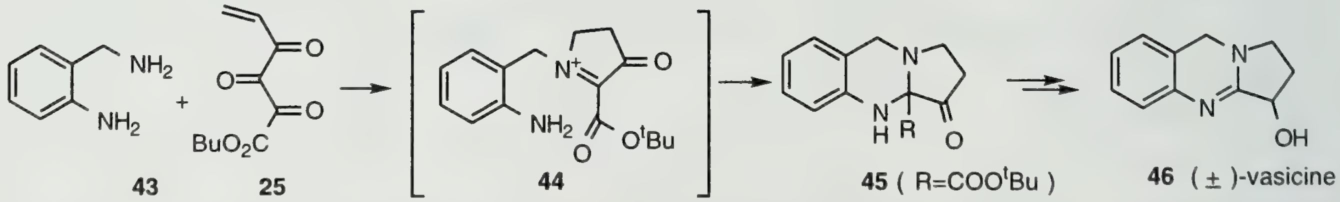
In the synthesis of tricyclic lactam **42** (Scheme VIII), the salt **41** was formed from the reaction of amino amide **39** with compound **40**. In the third stage reaction the amide residue added to the iminium salt to form β-lactam **42**.[22]

Scheme VIII



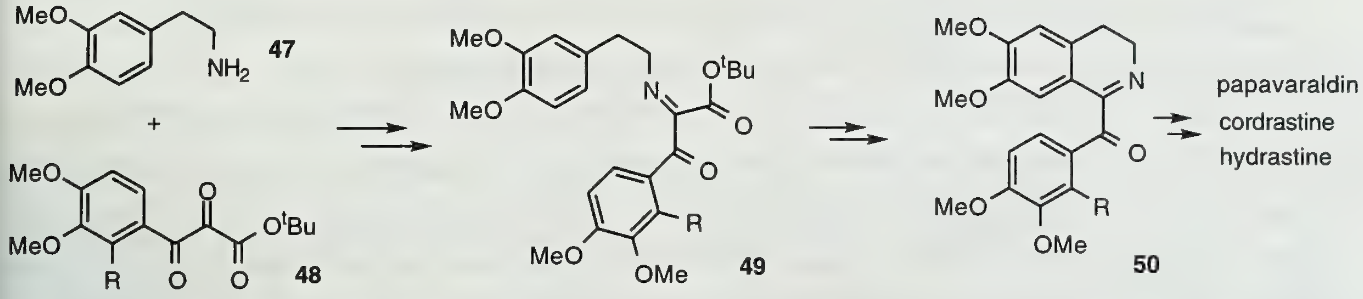
In the synthesis of vasicine, **46**, which displays strong hypotensive and respiratory stimulant activity, reaction of 2-aminobenzylamine (**43**) with the vinyl vicinal tricarbonyl reagent **25** in the presence of silica gel directly formed tricyclic **45**. In the absence of silica gel, the carbinolamine intermediate was isolated. The mechanism for the formation of **45** is proposed to go through iminium salt **44**. Further manipulation of compound **45** yielded vasicine, **46** (Scheme IX).[24]

Scheme IX



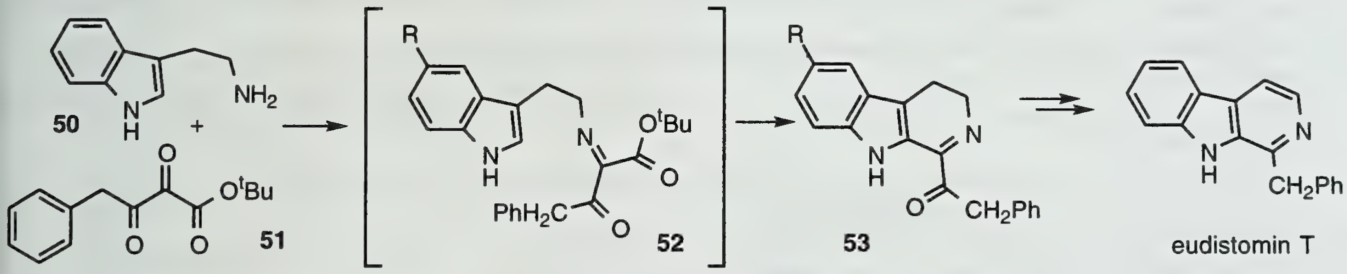
In the reaction of primary amine **47** with α,β-diketo ester **48**, the amino group of added twice to the central carbonyl to form **49**, and electrophilic addition at the benzene ring afforded the keto-imine **50**. This intermediate could be converted to papaveraldine, cordrastine, and hydrastine (Scheme X).

Scheme X



The same methodology was applied to the synthesis of eudistomins.[26] Stirring of tryptamine **50** with α,β-keto ester **25** in the presence of trifluoroacetic acid afforded the dihydro β-carboline **53**, most likely involving imine **52** as an intermediate. Carboline **53** was used in the synthesis of eudistomins T, I, and M, members of a family of marine alkaloids possessing antiviral activity (Scheme XI).[26]

Scheme XI



Conclusion

Recent discovery of tricarbonyls as binding units in FK-506 and rapamycin has rekindled interest in tricarbonyl chemistry. Recent progress toward the efficient synthesis of tricarbonyls in the presence of other functional groups has made these compounds more readily available. The highly electrophilic nature of the central carbonyl and the multiple electrophilicities of the structure have made tricarbonyl compounds an attractive functionality in synthesis. As the range of functional groups that can be attached to tricarbonyl units expands, more applications involving this moiety in synthesis can be expected.

References

(1) (a) R. de Neufville; H. von Pechmann *Chem. Ber.* **1890**, *23*, 3375. (b) Sachs, F.; Barschall, H.*Chem. Ber,* **1901**, *34*, 3047. (c) McCaldin, D. J. *Chem. Rev.* **1960**, *60*, 39.

(2) Rubin, M. B. *Chem. Rev.* **1975**, *75*, 177.

(3) (a) Tanaka, H.; Kuroda, A.; Marusawa, H.; Hatanaka, H.; Kino, T.; Goto, T.; Hashimoto, M. *J. Am. Chem. Soc.* **1987**, *109*, 5031. (b) Duyne, G. D. V.; Standaert, R. F.; Karplus, P. A.; Schreiber, S.L.; Clardy, J. *Science* **1991**, *252*, 839.

(4) (a) Findlay, J. A.; Radics, L. *Can. J. Chem.* **1980**, *58*, 579. (b) Albers, M. W.; Walsh, C. T.; Schreiber, S. L. *J. Org. Chem.* **1990**, *55*, 4984.

(5) Wasserman, H. H.; Pickett, J. E. *J. Am. Chem. Soc.* **1982**, *104*, 4695.

(6) Batchelor, M. J.; Gillespie, R. J.; Golec, J. M.; Hedgecock, C. J. R. *Tetrahedron Lett.*, **1993**, *34*, 167.

(7) Mahran, M. R.; Abdou, W. M.; Sidky, M. M.; Wamhoff, H. *Synthesis* **1987**, 506.

(8) Wilson, R. M.; Hengge, A. C. *J. Org. Chem.* **1990**, *55*, 197.

(9) (a) Hoffman, R. V.; Wilson, A. L.; Kim, H. O. *J. Org. Chem.* **1990**, *55*, 1267. (b) Hoffman, R. V.; Kim, H. O.; Wilson, A. L. *J. Org. Chem.* **1990**, *55*, 2820. (c) Hoffman, R. V.; Huizenga, D. J. *J. Org. Chem.* **1991**, *56*, 6435.

(10) Inokuchi, T.; Torii, S.; Liu, P. *Chem. Lett.* **1994**, 1411.

(11) (a) Schank, K.; Lick, C. *Synthesis* **1983**, 392. (b) Wasserman, H. H.; Han, W. T. *Tetrahedron Lett.* **1984**, *25*, 3743. (c) Saba, A. *Syn. Commun.* **1994**, *24*, 695. (d) Wasserman, H. H.; Vu, C. B.*Tetrahedron Lett.* **1990**, *31*, 5205.

(12) (a) Cooke, Jr. M. P.; Burman, D. L. *J. Org. Chem.* **1982**, *47*, 4955. (b) Wasserman, H. H.; Ennis, D.S.; Blum, C. A.; Rotello, V. M. *Tetrahedron Lett.* **1992**, *33*, 6003. (c) Wasserman, H. H. *J. Org. Chem.* **1995**, *60*, 8231.

(13) (a) Jones, T. K.; Mills, S. G.; Reamer, R. A.; Askin, D.; Desmond, R.; Volante, R. P.; Shinkai, I. *J. Am. Chem. Soc.* **1989,** *111*, 1157. (b) Jones, T. K.; Reamer, R. A.; Desmond, R.; Mills, S. G. *J. Am. Chem. Soc.* **1990**, *112*, 2998.

(14) Nakatsuka, M.; Ragan, J. A.; Sammakia, T.; Smith, D. B.; Uehling, D. E.; Schreiber, S. L. *J. Am. Chem. Soc.* **1990**, *112*, 5583.

(15) Nicolau, K. C.; Piscopio, A. D.; Bertinato, P.; Chakraborty, T. K.; Minowa, N.; Koide, K. *Chem. Eur. J.* **1995**, 318.

(16) (a) Williams, D. R.; Benbow, J. W. *J. Org. Chem.* **1988**, *53*, 4643. (b) Wasserman, H. H.; Rotello, V.M. *J. Org. Chem.* **1989**, *54*, 2785.

(17) (a) Danishefsky, S. J.; Egbertson, M. *J. Org. Chem.* **1989**, *54*, 12. (b) Linde II, R. G.; Jeroncic, L. O.; Danishefsky, S. J. *J. Org. Chem.* **1991**, *56*, 2534.

(18) Wasserman, H. H.; Baldino, C. M.; Coats, S. J. *Aldrichchim. Acta* **1987**, *20*, 63 and references there in.

(19) Wasserman, H. H.; Rotello, V. M.; Krause, G. B. *Tetrahedron Lett.* **1992**, *33*, 5419.

(20) Wasserman, H. H.; Lombardo, L. J. *Tetrahedron Lett.* **1989**, *30*, 1725.

(21) (a) Wasserman, H. H.; Kuo, G. H. *Tetrahedron* **1992**, *48*, 7071. (b) Wasserman, H. H.; Cook, J. D.; Vu, C. B. *J. Org. Chem.* **1990**, *55*, 1701.

(22) (a) Wasserman, H. H.; Henke, S. L.; Luce, P.; Nakanishi, E.; Schulte, G. *J. Org. Chem.* **1990**, *55*, 5821. (b) Wasserman, H. H.; Henke, S. L.; Luce, P.; Nakanishi, E.; Schulte, G. *J. Org. Chem.* **1992**, *57*, 2641.

(23) Wasserman, H. H.; Cook, J. D.; Vu, C. B. *Tetrahedron* **1992**, *48*, 2101 and references there in.

(24) Wasserman, H. H.; Kuo, G. H. *Tetrahedron Lett.* **1991**, *32*, 7131.

(25) Wasserman, H. H.; Amici, R.; Frechette, R.; van Duzer, J. H. *Tetrahedron Lett.* **1989**, *30*, 869.

(26) Wasserman, H. H.; Kelly, T. A. *Tetrahedron Lett.* **1989**, *30*, 7117.

(27) (a) Wasserman, H. H.; Han, W. T. *J. Am. Chem. Soc.* **1985**, *107*, 1444. (b) Wasserman, H. H.; Han, W. T. *Tetrahedron Lett.* **1984**, *25*, 3743.

(28) (a) schank, K.; Schuhknecht, C. *Chem. Ber.* **1982**, *115*, 3032. (b) Schank, K.; Lick, C. *Chem. Ber.* **1982**, *115*, 3890. (c) Schank, K.; Schuhknecht, C. *Chem. Ber.* **1982**, *115*, 2000.

(29) Schank, K.; Lick, C. *Synthesis* **1983**, 392.

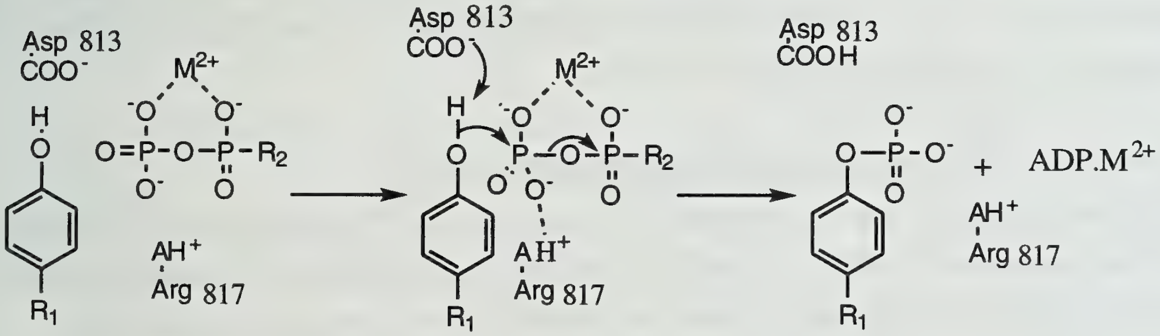
(30) (a) Saba, A. *Synth. Commun.* **1994**, *24*, 694. (b) Detering, J.; Martin, H. -D. *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 695.

INHIBITION OF EPIDERMAL GROWTH FACTOR RECEPTOR TYROSINE KINASE (EGF-RTK)

Reported by Wang Mao March 18, 1996

INTRODUCTION

Cell-surface receptor tyrosine kinases (RTKs) are crucial components in the signal transduction system that regulates the development of multicellular organisms. RTKs catalyze the phosphorylation of exogenous substrates as well as tyrosine residues within their own polypeptide chains, a process known as the autophosphorylation.[1] After activation by binding to an external growth factor, RTKs catalyze the transfer of the terminal phosphate from ATP to the phenolic OH group of a tyrosine residue in a substrate protein (the general mechanism of tyrosine phosphorylation catalyzed by EGF-RTK is shown in Scheme 1). In this way, the extracellular message can be translated across the membrane, and the intracellular catalytic cascade then mediates a variety of cellular responses. These activities include cytokine responses, antigen-dependent immune responses, cellular transformation by RNA viruses, regulation of the cell cycle, oncogenesis, and the modification of cell morphology.[2,3] The over-expression of RTK activity can result in the unregulated cell proliferation associated with cancer and other malignancies. The inhibition of tyrosine kinase activity may modulate the uncontrolled cellular proliferation and provide an approach to antitumor therapy. Some natural products such as erbstatin[4] have been found to possess antitumor activity by inhibiting the epidermal growth factor receptor tyrosine kinase (EGF-RTK). These natural products are a basis for the design of the EGF-RTK inhibitors.



Scheme 1

Copyright © 1996 by Wang Mao

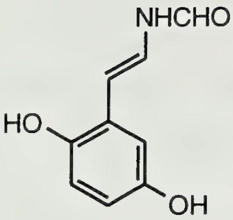
STRUCTURAL CHARACTERISTICS AND AUTOPHOSPHORYLATION

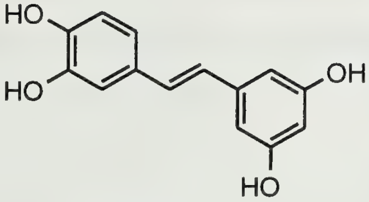
Epidermal growth factor receptor (EGF-R) is a 170 kDa RTK which consists of an extracellular ligand-binding domain linked by a hydrophobic transmembrane helix, a catalytic domain possessing an ATP binding site, a protein substrate binding site, and a C-terminal tail which contains several tyrosine phosphorylation sites.[6] By competing for the substrate binding sites, the autophosphorylation sites in the C-terminal tail of EGF-R behave as modulators of EGFR-substrate interactions. Autophosphorylation of the C-terminal tail decreases this domain's ability to act as a competitive inhibitor of substrate binding. As a result, protein substrates have an unhindered approach to the substrate binding sites.[7] The binding of the EGF causes a conformational change of the extracellular domain which is believed to induce dimerization of the receptor.[1] Dimerization stabilizes interactions between adjacent cytoplasmic domains and activates autophosphorylation.[5] In turn, this establishes a conformation of the receptor competent to interact and phosphorylate specific cellular substrates (e.g. the SH2 domain in Grb2) and initiate the signal cascade.[8] The effective inhibition of autophosphorylation will completely shut down such a signaling pathway.

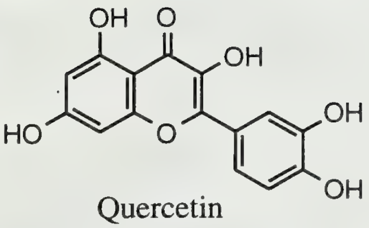
The catalytic domain of RTKs is highly conserved. The ATP binding site exhibits an even higher amino acid sequence homology, including a highly conserved lysine residue (Lys 721 in human EGF-R), a Gly X Gly XX Gly consensus sequence at the N-terminal end of the catalytic region, and a tyrosine (Tyr 845 in human EGF-R).[1] Kinetic analysis reveals that the EGF-RTK inhibitors can be grouped as the competitive inhibitors of ATP, competitive inhibitors of the protein substrate, or associative competitive inhibitors of both. Due to the high sequence conservation of the ATP binding site and the high ATP concentration in cell, the ATP competitive inhibitors are believed to have a broad-spectrum selectivity, and to be less effective *in vivo* than in *in vitro* assays. Substrate competitive inhibitors would also display reduced effectiveness, because the substrate concentration is high as a result of the proximity effect in the autophosphorylation reaction. Thus, the improvement of the potency and the selectivity of inhibitors are two major challenges in the design of EGF-RTK inhibitors.

NATURAL PRODUCTS BASED SYNTHESIS INHIBITORS

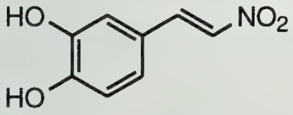
Several natural compounds such as erbstatin (**1**), piceatannol (**2**),[9] quercetin (**3**),[10] genistein,[11] lavendustin A[12] and staurosporin (**10**)[13] are tyrosine kinase inhibitors possessing micromolar activities. These compounds have provided the structural basis for the design of new tyrosine kinase inhibitors.

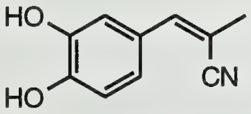

Erbstatin
1

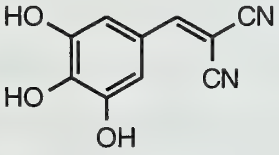

Piceatannol
2

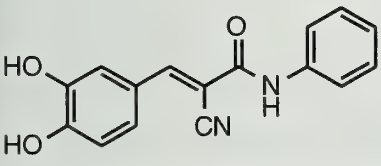

Quercetin
3

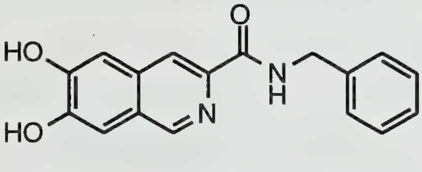
Erbstatin is a competitive inhibitor of the protein substrate, which inhibits EGF-R and src (a non-receptor protein with tyrosine kinase activity). Piceatannol inhibits p40 and p56^{lck} (two non-receptor tyrosine kinases). Erbstatin and piceatannol possess a phenolic styrene subunit which is a common structural feature in this class of inhibitors. Some simple derivatives, such as **4**, also show inhibitory activity of EGF-R (IC_{50} =8.8 μM). Levitzki and coworkers suggested that the 3,4-dihydroxybezene ring is essential for this series of inhibitors and a nitrile group is required to be in a *cis* and coplanar orientation.[14] The constrained bicyclic analogue (**8**) of the cinnamamide (**7**) shows high specificity against EGF-R (IC_{50} =3.1 μM for EGF-R; IC_{50} > 100 μM for p56^{lck}), while some other isoquinoline derivatives show selectivity against p56^{lck}.[15] Compared with erbstatin (IC_{50}=2.0 μM), the derivatives show little improvement in inhibition potency but are more selective than the parent compound. Enhancement of selectivity remains a task in this area. For example, the difference of AG213's (**9**) inhibition potency for EGF-R (IC_{50} =0.8 μM) and Ins-R (IC_{50} =640 μM) is a factor of 800, but it is only a factor of 8 when EGF-R is compared *in vitro* with PDGF-R (platelet-derived growth factor receptor, an RTK in subclass III) and p210$^{Bcr\text{-}Abl}$ (a non-receptor tyrosine kinase).[14]

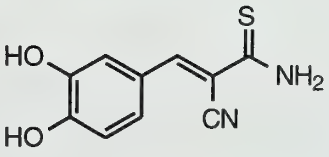

4


5

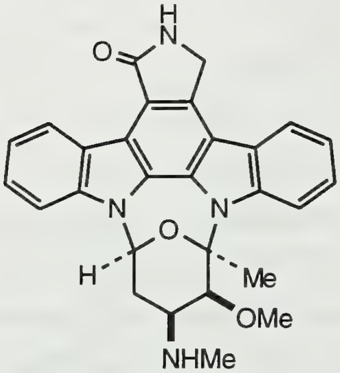

6 AG82


7 AG494


8

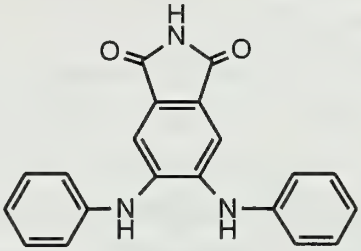

9 AG213

Staurosporin (**10**), which possesses a bis-indole alkaloid backbone, is a potent inhibitor of PKC (protein kinase C) and RTKs. Interestingly, its derivatives (e.g. **11**, **12**) show different selectivity for EGF-R and PKC, which is explained by the conformational difference between their structures: the staurosporine aglycone (SA) is flat, while DAPH 1 adopts a propeller-shaped conformation.[15]



Staurosporin

10

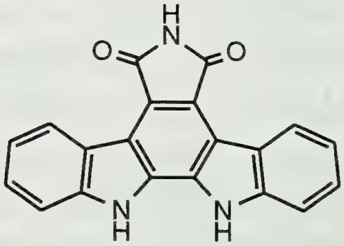


DAPH 1

11

EGF-R PKC

IC$_{50}$ 0.3μM 80μM



SA

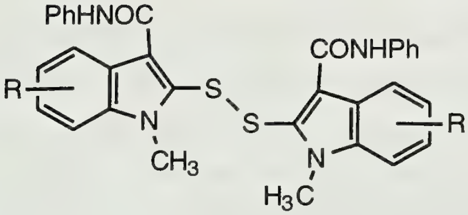
12

EGF-R PKC

IC$_{50}$ >100μM 0.31μM

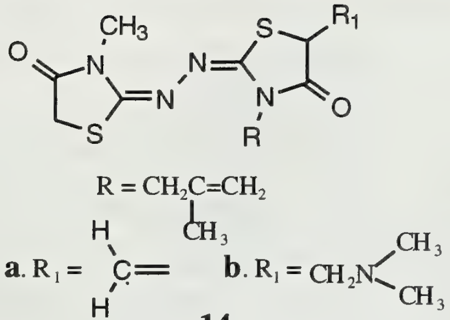
Another class of bis-indole EGF-RTK inhibitors is illustrated by 2,2'-dithiobis (1-methyl-N-phenyl-1-*H*-indole-3-carboxamide) (**13a**) which inhibits $pp60^{v\text{-}src}$ (IC$_{50}$=3.2 μM) and EGF-R (IC$_{50}$=10 μM). Tautomerism of thione-thiol is believed essential for inhibition and the dimers show more activity than the corresponding monomers. Most derivatives with electron-withdrawing groups are inhibitory, but 4-substitution results in inactive compounds. Side chains, such as 3-(N-benzylpropanamide) and 3-(N-phenyl carboxamide), which can provide hydrogen-bonding sites and lipophilic character, can improve EGF-RTK inhibition. Some derivatives (e.g. **13b, c**) show good selectivity between these two enzymes (Table 1).[17] The kinetic analysis shows that most of the compounds in this series are noncompetitive protein substrate and ATP inhibitors.[18]

Thiazolidine-diones (**14**) also show inhibitory activity against the EGF-R and c-src.[19] This class of compounds inhibit EGF-dependent cell proliferation effectively than they inhibit EGF-R tyrosine kinase. This could be due to the activity of other phosphorylation substrates downstream in the EGF signaling pathway which are more sensitive than the autophosphorylation substrates.



a. R = H
b. R = 5-Cl
c. R = 6-OAc

13

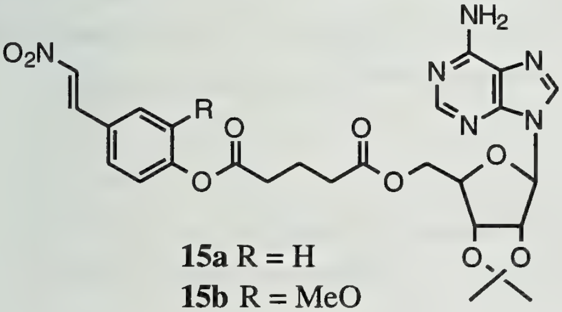


14

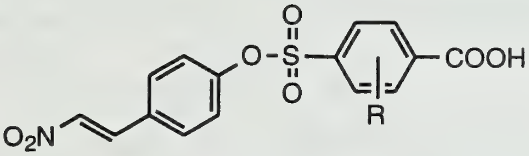
Mechanism Based Inhibitors

Rate studies suggest that EGF-RTK forms a ternary complex with the ATP and the peptide substrate. A bell-shaped pH dependent relationship of EGF-RTK activity together with the measurement of the enthalpy of ionization imply that in the EGF-RTK, Asp 813 behaves as a catalytic base and Arg 817 as a catalytic acid (Scheme 1).[20]

Early designs of mechanism-based inhibitors include adenosine 5'-glutarate (**15**)[21] and sulfonylbenzoyl nitrostyrene (**16**)[22] derivatives that mimic the transition state of tyrosine phosphorylation. These compounds were based on a model derived from an early NMR NOE measurement of c-AMP-dependent protein kinase (cAPK), which suggested that the distance between serine oxygen and ATP γ-phosphate was 5.3 Å.[23a] Subsequent crystallographic analysis of cAPK showed that this distance is only *ca.* 3 Å.[23b] Thus, it is not surprising that only limited improvement was observed in these compounds (e.g. **15** & **16**), since the molecular design relied on an incorrect interatomic distance.

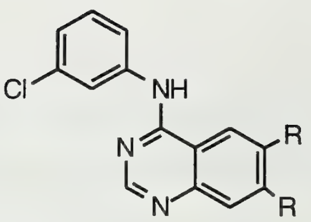


15a R = H
15b R = MeO

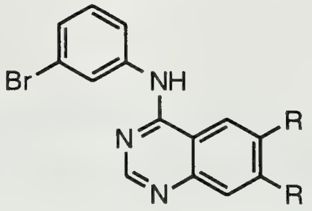


16a R = 2-OH
16b R = 3-OH

Due to the high sequence homology and presumable structure of tyrosine kinases, the crystal structure of the tyrosine kinase domain of insulin receptor can be used as a model of the EGF-R tyrosine kinase domain. In the active site of insulin receptor, Asp 1,132 (Asp 813 in EGF-R) is the catalytic base, Arg 1,136 (Arg 817 in EGF-R) provides the electrophilic catalysis and Asn 1,137 (Asn 818 in EGF-R) is hydrogen-bonded to Asp 1,132 O and also participates in M^{2+} coordination (usually Mg^{2+} or Mn^{2+}).[24]

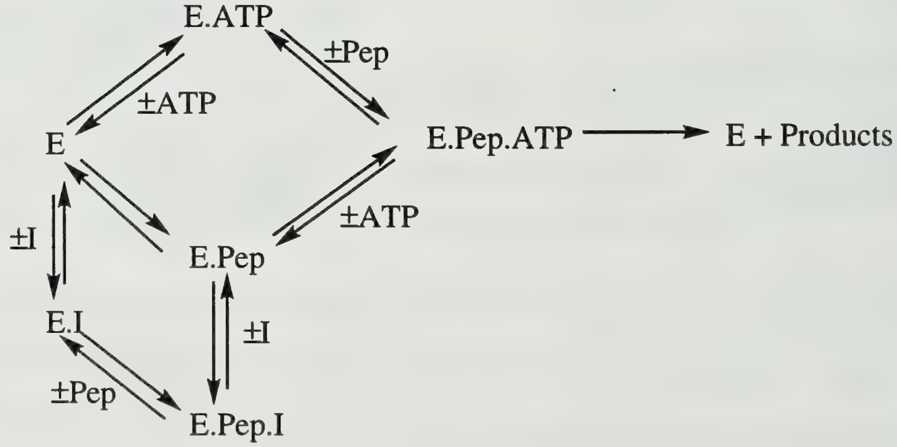


17a CAQ R = H
IC_{50} = 40 nM for EGF-R
17b AG1478 R = OMe
IC_{50} =0.31 nM for EGF-R



18a PD153035 R = OMe
IC_{50} = 0.025 nM for EGF-R
18b R = OEt
IC_{50} = 0.006 nM for EGF-R

Based on the proposed transition state of tyrosine phosphorylation, Ward and coworkers carried out a three-dimensional search on the ZENECA Core Collection. The structural features searched were the substitutes on the hydrophobic tyrosyl aromatic ring, tyrosyl phenolic oxygen, and the non-bridging oxygens of the ATP γ-phosphate. A new class of anilinoquinazoline EGF-R tyrosine kinase inhibitors (**17a, b**) was identified by this method.[20, 25] Potencies are very high, and **17b** showed good selectivity for EGF-RTK (Table 1). The anilinoquinazoline matches the transition state of tyrosine phosphorylation well: the anilino aromatic ring corresponds to the tyrosine aromatic ring, the anilino nitrogen represents the tyrosyl phenolic oxygen, and the quinazoline nitrogens behave as the oxygens in the ATP γ-phosphate. A kinetic analysis reveals that anilinoquinazoline derivatives are competitive with ATP and noncompetitive with protein substrate, even though they were designed as bisubstrate inhibitors. A possible kinetic mechanism that accounts for all these observations is shown in Scheme 2.[20] Further studies found that **18a** and **18b** have extraordinary potency, especially the latter whose IC_{50} is 0.006 nM. This represents the most potent EGF-RTK inhibitor reported to date.[26] The mode of the action of these compounds has yet to be established, but is thought to result from an induced conformational change in the enzyme.



Scheme 2

CONCLUSION

Several classes of compounds derived from natural products have been studied as EGF-RTK inhibitors. Many derivatives show higher selectivity and potency than their parent compounds, and provide valuable information about the molecular and cellular mechanisms involved in the tyrosine kinase catalytic procedure. Mechanism-based screening has identified several classes of inhibitors with high potency and selectivity, such as anilinoquinazolines. These results demonstrate that, even though the ATP binding site is highly homologous in kinase families, there still exist sufficient structural differences to allow for the design of selective ATP competitive inhibitors.

The improvement of the selectivity of EGF-RTK inhibitors is still a challenge. Some questions, such as the origin of potency and selectivity of the inhibitors, still remain unanswered. Further studies should provide more accurate structural information regarding EGF-R, and enhance the rational design of more selective higher potency EGF-RTK inhibitors.

Table 1. Inhibitors' Selectivity

Inhibitors	PTKs and IC_{50} (μM)							
	EGFR	HER2-Neu	PDGFR	p210$^{Bcr-Abl}$	InsR	Src	PKC	cAPK
4	8.8	--	--	>100	--	--	>500	--
6	3	--	--	3.6	--	--	--	--
7	0.7	42	6	75	>100	--	--	--
9	0.8	--	3	6	640	--	--	--
13a	10	--	--	--	--	3.2	--	--
13b	4.3	--	--	--	--	>100	--	--
13c	>100	--	--	--	--	3.5	--	--
14a	1	--	--	~100	--	3	>100	>500
14b	2	--	--	>100	--	7	350	>500
15a	0.66	--	--	>100	--	>100	95	--
15b	0.6	--	--	>100	--	>100	78	--
16a	0.054	--	--	27	--	--	500	--
16b	0.31	--	--	~100	--	--	>500	--
17b	0.0003	>100	>100	>50	--	--	--	--

HER2-Neu: a RTK in subclass I. InsR: insulin receptor.

REFERENCES

(1) Yarden, Y.; Ullrich, A. *Annu. Rev. Biochem* **1988** *57*, 443.

(2) Shokat, K. M. *Chem. & Biology* **1995** *2*, 509.

(3) a) Ullrich, A.; Coussens, L.; Hayflick, J. S.; Dull, T. J.; Gray, A. et al. *Nature*, **1984** *309*, 418 25.b) Yamamoto, T.; Nishida, T.; Miyajima, N.; Kawai, S.; Ooi, T. et al. *Cell*, **1983** *35*, 71-78. c)Downward, J.; Yarden, Y.; Mayes, E.; Scrace, G.; Totty, N. et al. *Nature*, **1984** *307*, 521-27.

(4) a) Umezawa, H. et al. *J. Antibiot.* (Tokyo) **1986** *39*, 170. b) Imoto, M. et al. *Biochem. Int.* **1987** *15*, 98.

(5) Ullrich, A.; Schlessinger, J. *Cell*, **1990** *61*, 203-212.

(6) Fantl, W. J.; Johnson, D. E.; Williams, L. T. *Annu. Rev. Biochem.*, **1993** *62*, 453.

(7) a) Honegger, A.; Dull, T. J.; Bellot, F.; Van Obberghen, E. et al. *EMBO J.*, **1988**, *7*, 3045. b) Honegger, A. et al. *EMBO J.*, **1988** *7*, 3053.

(8) Gishizky, M. L. *Annu. Rept. Med. Chem.***1995** 247.

(9) Geahlen, R. L.; McLaughlin, T. L. *Biochem. Biophys. Res. Commun.* **1989** *165*, 241.
(10) Graziani, Y. et al. *Biochim. Biophys. Acta.* **1981**, *714*, 415.
(11) Akyama, T. et al. *J. Biol. Chem.* **1987**, *262*, 5592.
(12) Uehara, Y. et al. *Biochem. Int.* **1988**, *41*, 831.
(13) Furusaki, A.; Hashiba, N. et al. *J. Chem. Soc. Chem. Commun.* **1978**, 800.
(14) a) Yaish, P. et al. *Science* **1988**, *242*, 933. b) Gazit, A. et al. *J. Med. Chem.* **1989**, *32*, 2344. c) Gazit, A. et al. *J. Med. Chem.* **1991**, *34*, 1896. d) Gazit, A. et al. *J. Med. Chem.* **1993**, *36*, 3556.
(15) a) Burke, T. R. et al. *Bioorg. Med. Chem. Lett.* **1992**, *12*, 1771. b) Burke, T. R. et al. *J. Med. Chem.* **1993**, *36*, 425-432.
(16) Buchdunger, E. et al. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 2334.
(17) a) Denny, W. A. et al. *J. Med. Chem.* **1993**, *36*, 2459. b) Denny, W. A. et al. *J. Med. Chem.* **1994**, *37*, 598. c) Denny, W. A. et al. *J. Med. Chem.* **1994**, *37*, 2033. d) Denny, W. A. et al. *J. Med. Chem.* **1995**, *38*, 58.
(18) Kraker, A. J.; Jones, J. A.; Schemmel, M. E.; Moore, C. W. *Proc. Amer. Assoc. Cancer Res.* **1993**, *34,* 408.
(19) Lydon, N. B. et al. *J. Bio. Chem.* **1990**, *265*, 22255.
(20) Ward W. H. J.; Cook, P. N.; Slater, A. M.; Davies, D. H.; Holdgate, G. A.; Green, L. R. *Biochem. Pharma.* **1994**, *48*, 659.
(21) a) Peterli, S.; Stumpf, R.; Schweizer, M.; Sequin, U. *Helv. Chim. Acta.* **1992**, *75*, 696. b) Peterli, S.; Hubmann, D.; Sequin, U. *Helv. Chim. Acta.* **1994**,*77*, 59.
(22) Traxler P. M.; Wacker, O.; Bach, H. L.; Geissler, J. F. et al. *J. Med. Chem.* **1991**, *34*, 2328.
(23) a) Granot, J.; Mildvan, A. S.; Bramson, N. H.; Kaiser, E. T. *Biochem.* **1980**, *19*, 3537. b) Knighton, D. R.; Zheng, J.; Ten Eyck, L. F.; Xuong, N. -H.; Taylor, S. S.; Sowadski, J. M. *Science* **1991**, *253*, 414.
(24) Hubbard, S. R.; Wei, L.; Ellis, L; Hendrichson, W. A. *Nature* **1994**, *372*, 746.
(25) a) Barker, A. J.; Davies, D. H. **1992** European Patent, EP 520722-A. b) Barker, A. J. European Patent Application No. 0566 226 A1, Oct. 20, 1993.
(26) a) Bridges, A. J.; Denny, W. A. et al. *J. Med. Chem.* **1996**, *39*, 267. b) Thompson, A. M.; Bridges, A. J.; Fry, D. W.; Kraker, A. J.; Denny, W. A. *J. Med. Chem.* **1995**, *38*, 3780. c) Fry, D. W.; Kraker, A. J.; Mcmichael, A.; Bridges, A. J. et al. *Science* **1994**, *265*, 1093.

SYNTHESIS AND ACTIVITY OF THE ANTIFEEDANT: AZADIRACHTIN

Reported by Brice C. Nelson April 1, 1996

INTRODUCTION

The widespread use of toxic insecticides has become an increasingly important issue. Insect pests damage or destroy nearly one third of the world's food crop,[1] resulting in the loss of billions of dollars annually. Traditional synthetic insecticides (chlorocarbons, organophosphates, and carbonates) are toxic to a wide range of insects. Unfortunately, this toxicity often affects other species including humans (both directly and through concentration in the food chain).[2] As a result of widespread insecticide use, insecticide resistant strains of insects have evolved. Resistance compounds the problem by making higher concentrations or more toxic insecticides necessary.[1] The use of biodegradable antifeedants, specifically azadirachtin, has been proposed as a solution.[1] This abstract discusses the synthesis, biosynthesis, and activity of azadirachtin.

BACKGROUND

Natural products have seen limited use as effective alternatives to traditional synthetic insecticides. The natural products used in pest control are classified by their mode of action, as positive stimulus (pheromones) or negative stimulus (insect deterrents) agents. Due to their specificity and mode of action, pheromones can only be used as bait, in traps designed to capture a specific species (e.g., western pine beetle[3]). By contrast, insect deterrents are less specific and can be applied to a broad spectrum of species. The following five categories exist for the classification of insect deterrents.[4]

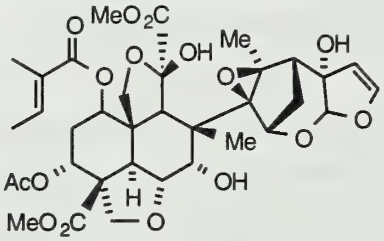
1. Repellents: repel insects from plants.
2. Suppressants: inhibit the initiation of feeding.
3. Deterrents: deter the continuation of feeding.
4. Antibiotics: interfere metabolically with growth development
5. Anorexigenics: produce a loss of appetite

An antifeedant is the most useful type of insect deterrent, because it displays activity in one or more of the above categories. Antifeedants are classified as primary, secondary, or in some circumstances both. Primary antifeedants exhibit characteristics from the first three categories, and secondary antifeedants exhibit characteristics of the latter two categories. Munkata defined antifeedants as "a chemical that inhibits feeding but does not kill the insect directly. The insect remains near the treated plant and dies from starvation."[5] This definition describes antifeedants with permanent effects; lower concentrations and less active antifeedants inhibit the insect temporarily. An ideal antifeedant would act temporarily, but make a plant so undesirable that an insect would eat the untreated weeds.[1]

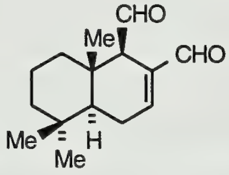
Copyright © 1996 by Brice C. Nelson

AZADIRACHTIN

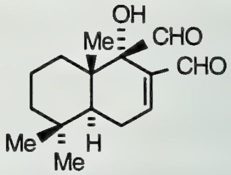
Azadirachtin (**1**) is the most effective antifeedant identified to date and has been the subject of several reviews.[6,7] Azadirachtin has a broad range of antifeedant activity and is also one of the three most potent feeding inhibitors, the others being warbungal (**2**) and polygodial (**3**).[1] Morgan first demonstrated azadirachtin's activity as an antifeedant in 1967.[8] Subsequent testing has proven that azadirachtin acts as an antifeedant in over two hundred species in the following orders: *Acarina* (mite), *Coleoptera* (beetle), *Diptera* (fruit fly), *Hemiptera* (June bug), *Homoptera* (aphid), *Hymenoptera* (ant), *Lepidoptera* (moth), *Orthoptera* (locust), and *Nematoda* (parasitic round worm).[9]



1



2



3

The Neem tree is the only known natural source of azadirachtin. Due largely to its medicinal properties, the Neem tree was propagated as a medicinal plant and has widespread growth in Africa and South America. Native to South Central Asia, the tree has recently been used in reforestation efforts. The Neem tree has shown good adaptability to different climates and grows in Florida, Oklahoma, Arizona, and California.[1] Due to its quick growth and abundant supply, the Neem tree is a convenient source of azadirachtin containing between 0.3 and 0.7 % by dry kernel weight. W.R. Grace and Co. patented Neem seed extract. The extract is sold under the trade name of Neemazad, and contains between 2500-3000 ppm of azadirachtin in the formulation.

Azadirachtin is superior to traditional insecticides. A growing plant absorbs and translocates the compound.[10] This means that azadirachtin protects parts of the plant that develop after application. By contrast, traditional synthetic insecticides only protect the applied surface. Azadirachtin is also nontoxic to humans, and, unlike traditional insecticides, many beneficial species including bees and spiders.[4] Neemazad™ passed all E.P.A. toxicity studies and is marketed as a "reduced risk pesticide."

In addition to its primary antifeedant properties, azadirachtin is also a secondary antifeedant,[11] acting as an antibiotic slowing growth and causing mutation. This activity is most notable in the disruption of the molting process, and a lowering of fecundity (capability of producing offspring). It also has anorexigenic properties causing insects to die of starvation. These secondary effects may prove to be more important than the primary effects, since they act at less than one tenth the concentration.

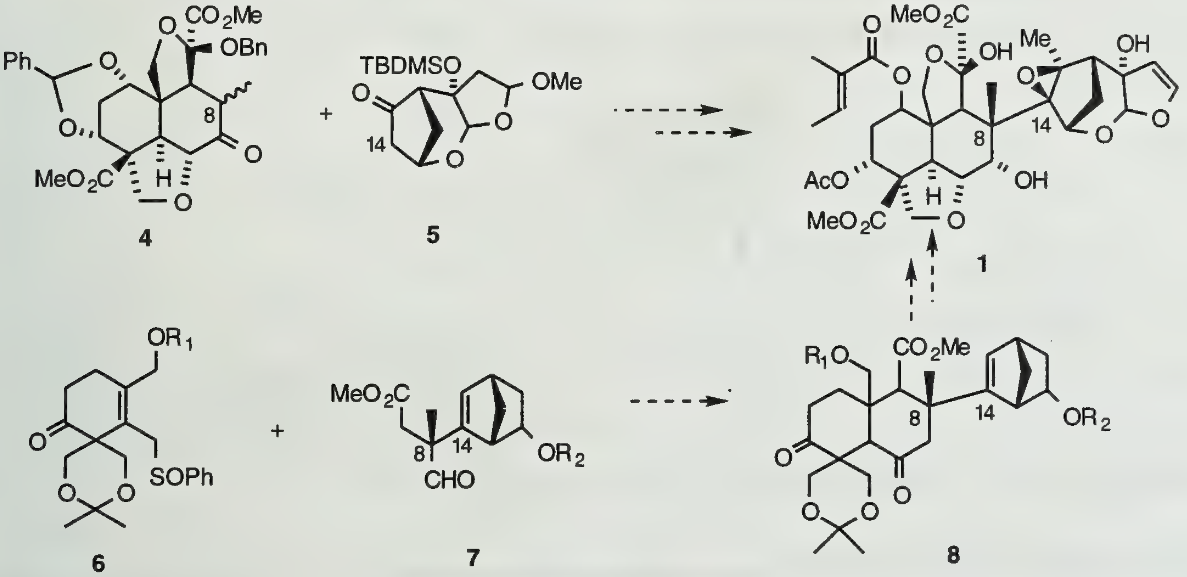
STRUCTURE

The structure of azadirachtin (**1**) was solved by NMR and X-ray crystallography of a derivative in 1987.[12] Azadirachtin ($C_{35}H_{44}O_{16}$) is a complex and interesting diterpene, possessing sixteen stereogenic centers, seven of which are quaternary. The molecule possesses a trans fused AB ring and a bicyclic dihydrofuran moiety. Azadirachtin is a highly oxidized compound containing an epoxide bridging carbons 13 and 14, three free hydroxyl groups (carbons 7, 11, and 20), and four ester linkages (carbons 1, 3, 4, and 11).

SYNTHESIS

Despite considerable effort, the total synthesis has yet to be achieved. Synthetic approaches by Ley and coworkers[13] as well as Shibasaki and coworkers[13] envision a convergent synthesis, consisting of a late coupling of the two halves (**4** and **5**) at the C8-C14 bond (Scheme I). The syntheses, of several precursors resembling **4** and **5** are complete, and coupling studies are currently in progress.[14]

Scheme I

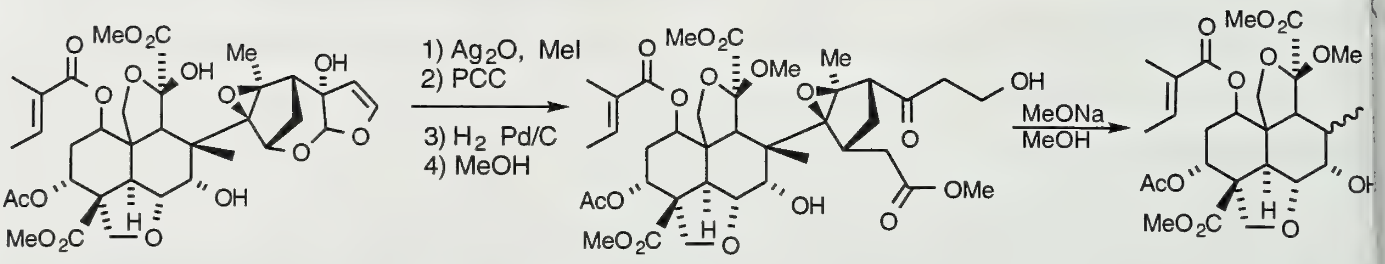


To avoid the difficulty of forming the C8-C14 bond, Mori and Watanabe[13] envision the coupling of compounds **6** and **7** forming the B ring in **8** (Scheme I). Subsequent manipulations will focus on the functionalization of the dihydrofuranacetal portion of azadirachtin. To date Mori and Watanabe have only published model studies.[15]

DEGRADATION

Ley and coworkers[6] have done extensive degradation studies on isolated azadirachtin. These studies have introduced a large literature of functional group manipulations, and more importantly two synthetic intermediate targets (**10** and **12**). As shown in Scheme II oxidative cleavage of azadirachtin produces compound **10**, which is similar to the synthetic left half (**4**). Compound **10** has been synthesized from starting materials, allowing connection of the research on the total synthesis and degradation of azadirachtin.[16]

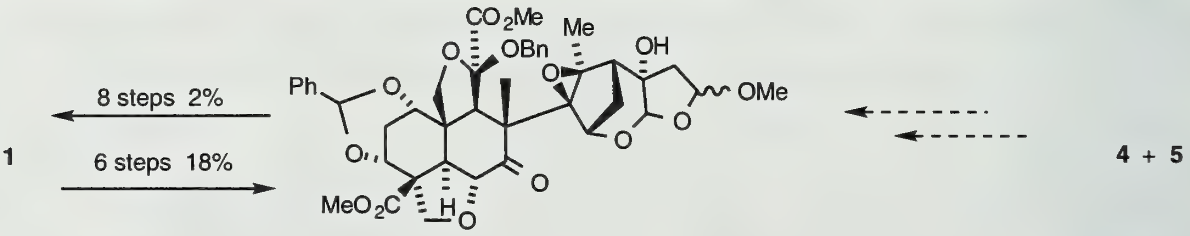
Scheme II



1 **9** **10**

Another compound formed by selective degradation is the advanced relay intermediate (**12**). Coupling of **4** and **5** by the proposed methodology of Ley or Shibasaki will produce compound **12** (Scheme III), thus linking the total synthesis of azadirachtin to available starting materials.[13] If this coupling can be accomplished the total synthesis is possible and absolute configuration can be proven. Compound **12** is obtained from azadirachtin in six steps with a 18% yield and converted back in eight steps with a 2% yield.

Scheme III

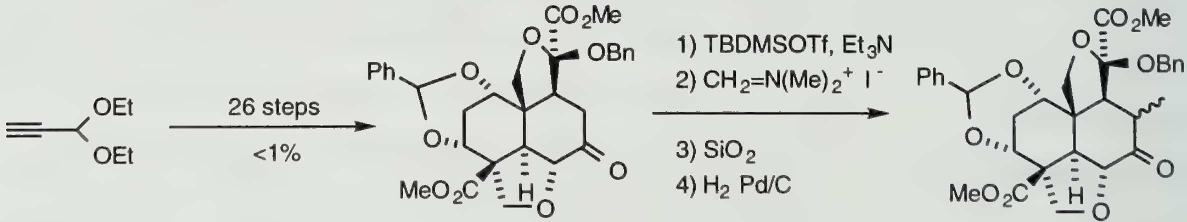


12

SYNTHESIS OF THE LEFT HALF BY LEY'S ROUTE

In the literature several methods of constructing a left half for either the Shibasaki or Ley coupling have been proposed.[6] Ley's synthesis involves a particularly efficient silicon directed intramolecular Diels Alder approach. His synthesis of the protected intermediate **14** (Scheme IV) proceeds from commercially available starting material in 26 steps with a yield of less than one percent.[17] Intermediate **14** was enolized and treated with Eschenmoser's salt to form the α-methylene group, which was subsequently reduced stereoselectively to produce **4** in a 4:1 ratio of β and α isomers (31% overall yield).[18] In the Ley or Shibasaki procedure either compound **14** or **4** may be suitable as the left hand fragment, dependent on steric constraints and relative reactivity.

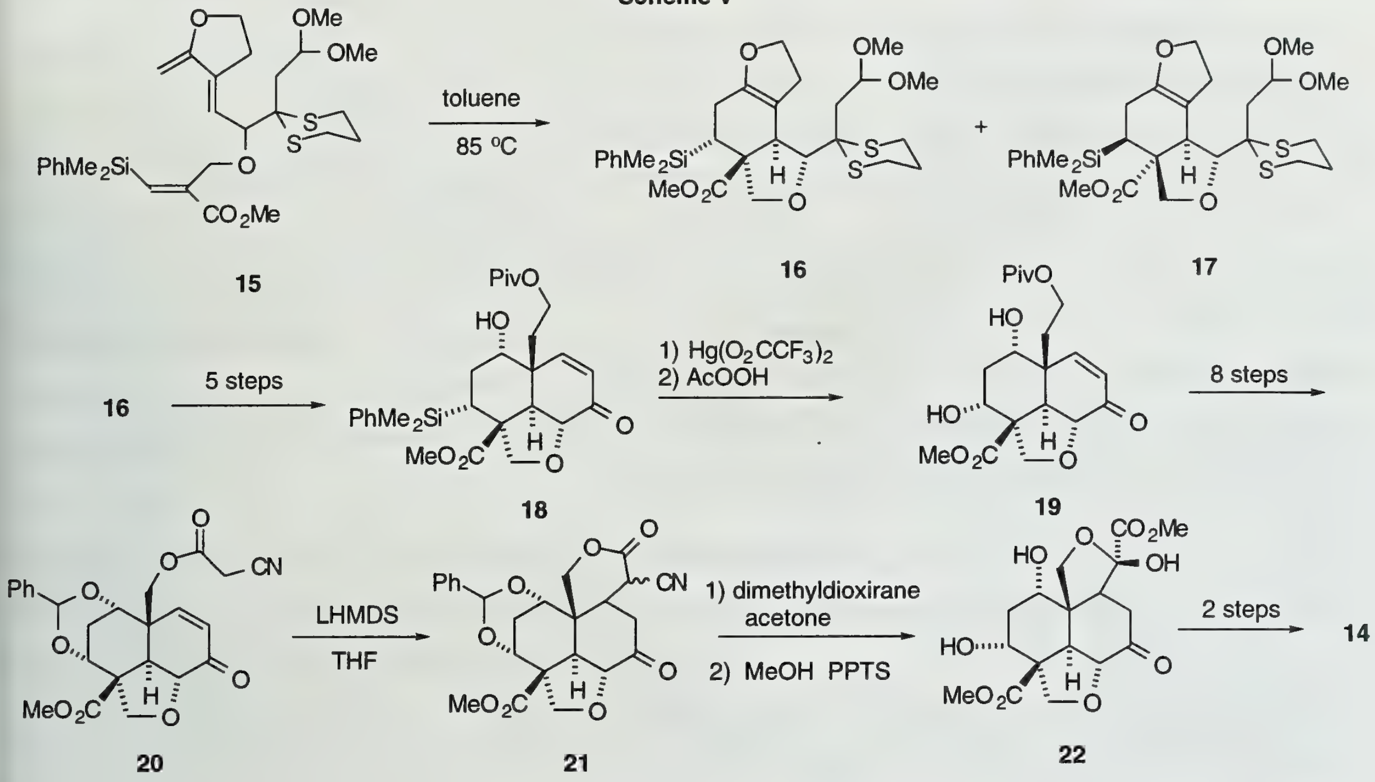
Scheme IV



13 **14** **4**

Ley's use of silicon in the synthesis of **14** (Scheme V) is interesting and has several noteworthy steps. The silyl group in **15**, which is sterically demanding, directs the intramolecular Diels Alder cyclization. The trans fused endo product **16** is favored over the cis fused exo isomer **17** by a 5:2 ratio. The silyl group is again utilized when oxidizing alcohol **18** to form the diol **19** via a silyl-Baeyer-Villiger oxidation.[18]

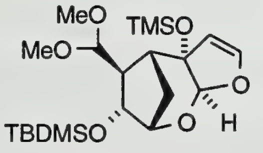
Scheme V



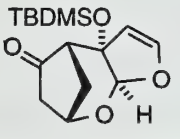
The tetrahydrofuran hemiketal **22** was formed from cyano acetate **20** by a Michael addition, oxidation, and subsequent methoxide initiated ring contraction. Compound **22** is transformed to **14** with two protection steps.

SYNTHESIS OF POTENTIAL RIGHT HALVES FOR A LEY OR SHIBASAKI COUPLING

Of the possible potential right half precursors, **23** and **5** are the most promising. Both syntheses are straight forward.



23



5

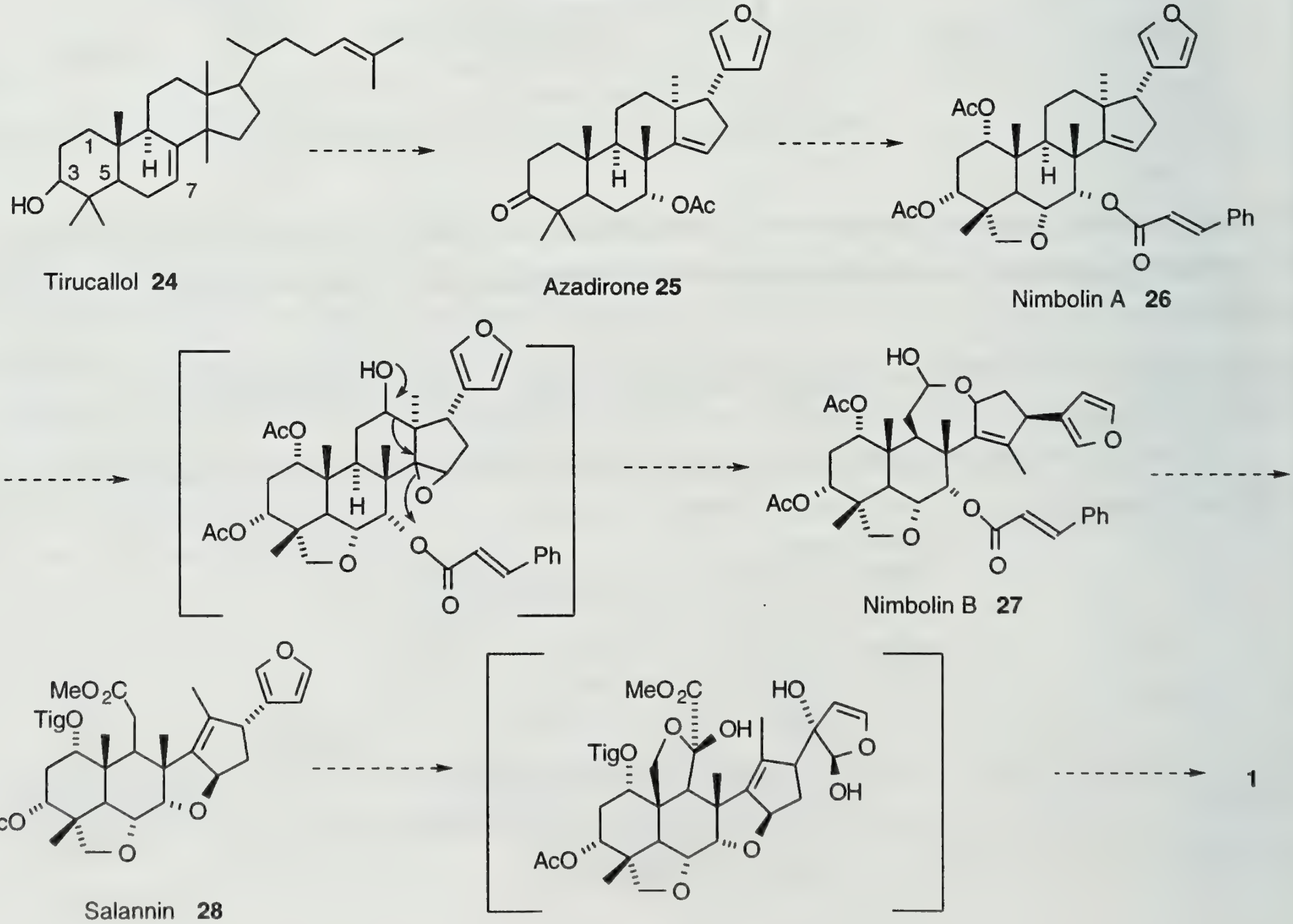
Precursor **23**, synthesized by Henry and Fraser-Reid from D-galactol in nine steps, is promising due to crystalline intermediates and a yield of 7.6%.[19] Ley and coworkers synthesized precursor **5**, from known starting material in 15 steps with an overall yield of 0.4%.[20]

BIOSYNTHESIS

The biosynthetic pathway may contain the key to azadirachtin's future. Once better understood, insights in utilizing the neem tree or a similar plant as a bulk producer of azadirachtin may be forthcoming.[21] Genetic manipulation of the plants in need of protection to produce their own azadirachtin is also a possibility and precedent for this has been established. Genetically modified tobacco, cotton, and corn plants produce an insecticide derived from the bacterium Bacillus thuringenesis.[22]

Azadirachtin is a limonoid (tetranortriterpene) with both the C and the D rings opened by oxidation. The probable biosynthetic pathway is outlined in Scheme VI.[6] All compounds have been isolated from the Neem tree, except Tirucallol (**24**). In vivo introduction of C^{14} labeled Tirucallol permitted isolation of labeled Nimbolin B (**27**).[1] The path from Tirucallol (**24**) to Nimbolin A (**26**) proceeds by oxidative cleavage of four side chain carbons and subsequent cyclization to form the furan moiety, as well as oxidative functionalization of carbons 1, 3, 4, 6, and 7. Nimbolin A (**26**) is transformed to Nimbolin B (**27**) by oxidation to a epoxide intermediate which undergoes oxidative cleavage and subsequent hemiacetal formation. Nimbolin B (**27**) is then transformed to Salannin (**28**) by oxidative hemiacetal opening, followed by displacement of the protonated 7 hydroxyl. Azadirachtin (**1**) is formed from Salannin (**28**) by a series of oxidations and rearrangements.

Scheme VI



STRUCTURE ACTIVITY RELATIONSHIPS

The total synthesis of azadirachtin will be a milestone, but commercial application of synthesized azadirachtin will be prohibitively expensive. Due to the many steps and low yields, efforts directed at determining the structural factors that govern the activity of azadirachtin are underway. The design and synthesis of simpler and/or more effective derivatives might be possible after elucidation of the structural features necessary for the antifeedant activity.

The literature contains a massive survey of precursors and derivatives of azadirachtin[6], but none are as effective as the parent compound over a broad range of insects. Azadirachtin affects a wide spectrum of insects, and different researchers have used many different species to test derivatives. This complicates comparisons because activity of the derivatives is species dependent. Inconsistency in the literature, caused by these variables, makes it difficult to derive any empirical relationships between structure and activity. Some data can be correlated and will be discussed below.

PRIMARY ANTIFEEDANCY

Primary antifeedancy interferes with an insect's chemoreception, which is the mechanism by which an insect determines if a compound is palatable. Chemoreception functions by cross-fiber patterning, utilizing several different types of receptor cells, which are located in the setae on antennae, feet, and/or mouth parts of insects. Since chemoreception involves the interaction of several receptor cells and not one particular receptor, structure activity analysis of primary antifeedancy is complicated. To date, the molecular basis for chemoreception is largely unknown.[21]

General conclusions about derivatives of azadirachtin that apply across the orders can be derived. Substitutions of increasingly large substituents on carbons 1 and 3 causes a rapid lose of activity. Hydrogenation of the C-22 double bond does not result in a loss of activity. The C-7, C-11, and C-20 hydroxyls, and the epoxide are all necessary for good activity, probably due to structure constraints. X-ray structure and variable temperature ^{1}H-NMR analyses indicate that azadirachtin adopts a rigid framework supported by hydrogen bonds. The hydroxyl group on C-11 forms a hydrogen bond with the epoxide and the hydroxyl on C-20 donates a hydrogen bond to the hydroxyl group on C-7.

Fragments similar to **4** and **5** exhibit antifeedant activity. The right half fragments are nearly twice as potent as the left, although both are considerably less active than azadirachtin. Interestingly, hydrogenation across the double bond, which cause no loss of activity in azadirachtin, has a significant effect on the activity of the right fragment. Combining a left fragment and a right fragment in the same assay gives a synergistic increase in antifeedant activity,[23] but this combination is still notably less active han azadirachtin.

SECONDARY ANTIFEEDANCY

In contrast to azadirachtin's primary effects, its secondary effects show less species dependent ctivity.[11] The mechanism of these effects is unknown and the subject of some debate and considerable research effort. Rembold has shown that these secondary effects are independent of azadirachtin's primary antifeedant activity.[24] A recently synthesized azadirachtin derivative, with a fluorescent dansyl group attached to the C-3 hydroxyl, exhibited high secondary activity when injected into insects.[25] The use of that derivative and other similarly labeled derivatives should be instrumental in determining the site of action, and ultimately the mechanism of secondary effects.

CONCLUSIONS

Azadirachtin is a good non-toxic alternative to current insecticides. Azadirachtin has good activity and broad applicability as well as the benefit of secondary antifeedant effects; making it the most attractive antifeedant known for agricultural use. More research is necessary to determine sites of action so isolation of proteins and cloning of genes is possible. The study of the receptor sites and/or enzymes affected, will allow a better understanding of structure activity relationships and possibly the syntheses of more effective and/or cheaper analogs.

REFERENCES

1. Jacobson, M. In *Focus on Phytochemical Pesticides,* CRC Press, 1988; Vol.1.
2. Waldholz, M. "Controversy Builds Over Threat of Common Chemicals" *The Wall Street Journal* 7 Mar. 1996.: 127:98.
3. Bedard, W.D.; Wood, D.L. "Programs Utilizing Pheremones in Survey and Control: Bark Beetles -the Western Pine Beetle" in *Pheremones*, M.C. Birch, North Holland, Amstrerdam, 1974.
4. Morgan, E. D.; Mandava, N. B. In *Handbook of Natural Pesticides*, CRC Press, 1990, Vol. 6.
5. Munkata, K. *Pure Appl. Chem.* **1975**, 57-66.
6. Ley, S.V.; Denholm, A.A.; Wood, A. *Natural Product Reports* **1993**, 109-157.
7. Subrahmanyam, B. *Proc. Indian Acad. Sci.* **1990**, 277-288.
8. Butterworth, J. H.; Morgan, E. D. *Chem. Comm.* **1968**, 23-24.
9. Green, M. B.; Hedin, P. A. In *Natural Resistance of Plants to Pests,* ACS Symposium Series 189; American Chemical Society: Washington D.C., 1985.
10. Gill, J.S.; Lewis, C.T. *Nature*. **1971**, 402-403.
11. Mordue, A.J.; Blackwell, A. *J. Insect Physiol.* **1993**, 903-924.
12. Kraus, W.; Bokel, M.; Bruhn, A.; Cramer, R.; Klaiber, I.; Klenk, A.; Nagl, G.; Pohnl, H.; Sadlo, H.; Vogler, B. *Tetrahedron.* **1987**, 2817-2830.
13. Denholm, A.A.; Jennens, L.; Ley, S.V.; Wood, A. *Tetrahedron*. **1995**, 6591-6604.
14. Ley, S.V. *Pure Appl. Chem.* **1994**, 2099-2102.
15. Pereira, O.Z.; Chan, T.H. *J. Org. Chem.* **1994**, 6710-6716.
16. Ley, S.V.; Lovell, P.J.; Slawin, A.M.Z.; Smith, S.C.; Williams, D.J.; Wood, A. *Tetrahedron,* **1993**, 1675-1700.
17. Kolb, H.C.; Ley, S.V.; Slawin, A.M.Z.; Williams, D.J. *J Chem. Soc. Perkin Trans. 1.* **1992**, 2735-2762.
18. Koot, W.; Ley, S.V. *Tetrahedron*, **1995**, 2077-2090.
19. Henry, K.J.; Fraser-Reid, B. *J. Org. Chem.* **1994**, 5128-5129.
20. Anderson, J.C.; Ley, S.V.; Santafianos, D.; Sheppard, R.N. *Tetrahedron*, **1991**, 6813-6850.
21. Allan, E.J.; Easwara, J.P.; Johnson, S.; Mordue, A.J.; Morgan, E.D.; Stuchbury, T. *Pestic. Sci.* **1994**, 147-152.
22. Vaeck, C. *Nature*. **1987**, 33-35.
23. Blaney, W.M.; Simmonds, M.S.J.; Ley, S.V.; Anderson, J.C.; Smith, S.C.; Wood, A. *Pestic. Sci.* **1994**, 169-173.
24. Rembold, H.; Sharma, G.K.; Czopplet, C.; Schmutterer, H. *Z. Angew. Entomol.* **1982**, 12-17.
25. Simmonds, M.S.J.; Blaney, W.M.; Grossman, R.B.; Ley, S.V. *J . Insect Physiol.* **1995**, 555-564.

ADDITIONS TO CARBONYL GROUPS IN AQUEOUS MEDIA

Reported by Timothy R. Wharton April 4, 1996

INTRODUCTION

Water is the most common solvent on Earth. It has only recently, however, seen use in standard organic reactions.[1] In fact, water holds several major advantages over conventional organic solvents. Water is plentiful, cheap, easily obtained, and has none of the toxicity and harmful environmental effects associated with organic solvents. Since most biochemical processes take place in an aqueous environment, most models or biomimetic models should be conducted in water. Perhaps most importantly, several organic reactions have shown distinct improvements in rate or selectivity when conducted in water. Specific examples of these enhancements will be presented in this abstract, with the emphasis on the chemistry of carbonyl compounds.

PROPERTIES OF WATER

Water is very polar, acts as both a hydrogen bond donor and acceptor, and is excellent at solvating ions and highly polar molecules. These properties make it quite different from organic solvents. Water can accelerate reactions by stabilizing polar intermediates or transition states.

The well-defined solution structure of water gives rise to another property, namely the hydrophobic effect.[2] Unlike most organic solvents, water forms a strong solution matrix, with molecules associated through hydrogen bonds. Any molecule dissolved in water must disrupt this network to some extent. Polar solutes compensate for this by forming favorable interactions with the water. Non-polar solutes, such as most organic molecules, cannot interact with the water in this fashion. These solutes tend to aggregate together to minimize disruption. Overall, non-polar molecules in water often behave as if they were under great pressure. Reactions that contract into their transition states will be accelerated, and, if there is a stereochemical or regiochemical option, the most compact transition state will be favored.

The hydrophobic effect is further affected by ionic species in solution.[3] Small ions, such as Li^+ and Cl^-, act as salting-out agents by "tightening" the aggregation of the solvent. This makes

Copyright © 1996 by Timothy R. Wharton

cavity formation less favorable, and increases the magnitude of hydrophobic effects.. Larger ions, such as guanidinium and perchlorate decrease hydrophobic effects by increasing the solubility of the non-polar solutes in water. These effects can be used as a probe for hydrophobic interactions. It is interesting to consider that hydrophobic acceleration is a direct result of the poor solubility of organic molecules in water, often thought of as an impediment to aqueous phase chemistry.

THE ALDOL CONDENSATION

The aldol condensation can be thought of as two separate reactions. The first is the addition of an enolate ion to a carbonyl compound to make a β-hydroxy ketone. The second reaction is the dehydration to produce the α,β-unsaturated carbonyl compound. The overall reaction is commonly done in an alcoholic solvent, and usually proceeds to the dehydrated product.

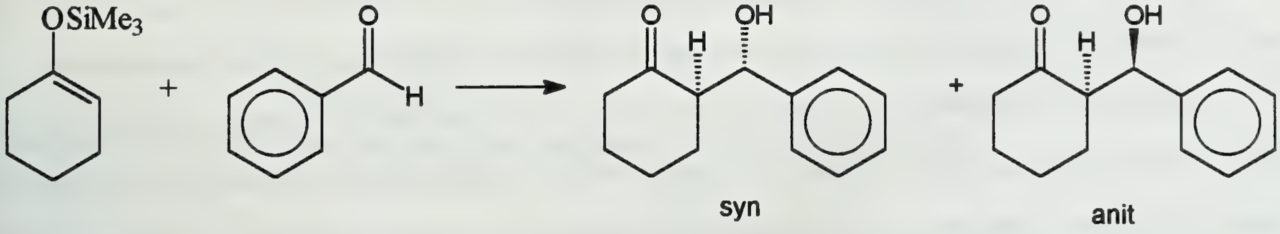
Fringuelli and co-workers studied the condensation reactions of acidic carbon compounds with benzaldehyde in basic aqueous solution in the presence of different surfactants.[4] They found that the reaction was catalyzed by cationic surfactants, such as $Bu_4N^+Br^-$. Surfactants capable of forming micelles further drove the reaction to dehydration, so more of the dehydrated product was recovered(Table 1). Presumably, the dehydration reaction proceeds more readily in the hydrophobic interior of a micelle. These reactions, like most aldol condensations, are conducted at fairly high pH and cause problems with base-sensitive substrates.

Table 1. Aldol condensations in water

	yields with various surfactants			
Ketone	**none**	**SLS**	**CTACl**	**TBACl**
acetophenone[a]	37/8	30/18	0/92	26/43
cyclohexanone[b]	91/9/0	82/18/0	0/0/90	84/12/4
isophorone[a]	24/0	--	0/80	27/58
$PhCH_2CN$	6	0	86	80
pNO_2-$PhCH_2CN$	7	0	85	36
$PhSO_2CH_2CN$	54	--	90	80

[a] Yields expressed as ketol/dehydrated products.
[b] Yields expressed as ketol/dehydrated/double products.

Aldol reactions are subject to Lewis acid catalysis, which lowers the pH necessary for the reaction to occur. In addition, the Lewis acid stabilizes the initial product, inhibiting the elimination reaction. Buonora and co-workers recently examined this phenomenon in greater detail.[5] They looked at the reaction between *p*-nitrobenzaldehyde and acetone while varying pH and the concentration of zinc nitrate and N,N-dimethyl-2-aminoethanol. The zinc nitrate without amino alcohol present lowers the pH required for addition but not for elimination. The addition of zinc nitrate and amino alcohol lowers the pH required for both addition and elimination. In this way, the range of pH values that promote addition but not elimination can be widened.



1 2 3 4

Scheme I. The Mukaiama reaction

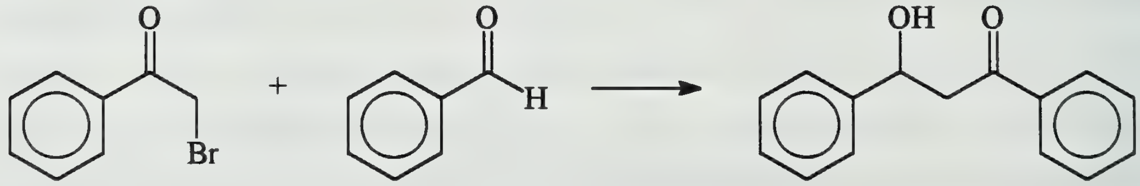
The Mukaiyama reaction[6] (scheme I) is a variant of the aldol reaction involving a silyl enol ether and an aldehyde, in the presence of a stoichiometric amount of a Lewis acid. Lubineau[7] has shown that this reaction can occur in aqueous solution without any acid or base catalysis, and often with the opposite stereochemical preference than observed in organic solvents. This stereochemical preference is also observed under high pressure,[8] indicating a possible hydrophobic effect. The anti product is formed from a thermodynamically more stable chair-like transition state, but the syn product comes from a more compact boat-like transition state, and should be favored under pressure.

Table 2. Mukaiyama reaction between **1** and **2** under different conditions

Solvent	**temperature**	**time**	**conditions**	**yield**	**syn:anti**
CH_2Cl_2	20	2 h	Lewis acid ($TiCl_4$)	82	25:75
CH_2Cl_2	60	9 days	10 Kbar pressure	90	75:25
H_2O	20	5 days	stirring or ultrasound	23	85:15
H_2O-THF	55	1 day	ultrasound	76	74:26
H_2O-DME	55	1 day	ultrasound	46	69:11
H_2O-CH_3OH	55	1 day	ultrasound	35	65:35
CH_3OH	55	1 day	ultrasound	30	57:43

The reaction is best conducted in a heterogeneous mixture of water and THF, using ultrasound to generate a fine emulsion. The yield and selectivity is reduced upon going to a more water-soluble co-solvent, presumably due to a greater solubility of the reactants and a reduction of hydrophobic effects. All of the starting materials reacted; and the low yields in some cases are due to premature hydrolysis of the silyl enol ether. The solvent effects also indicate that a hydrophobic acceleration is occuring. The neutral conditions are synthetically very useful, since acid or base sensitive groups do not cause a problem, and dehydration of the β-hydroxy ketone rarely occurs. The reaction has also been conducted with a range of aldehydes. Substituted benzaldehydes, formaldehyde, and acrolein all all reacted to give similar yields. Methyl vinyl ketone gave only 1,4 addition products in poor yields. With unconjugated ketones, no addition products were isolated.

Recently, Kobayashi and coworkers have shown that lanthanide triflates ($Ln(OTf)_3$) act as water-soluble Lewis acids,[9] allowing Lewis-acid catalyzed Mukaiyama reactions to take place in water. Ytterbium, gadolinium, and lutetium were found to give the best results. In the presence of these Lewis acid, Mukaiyama reactions proceed readily at room temperature and give excellent yields. Aldehydes used in the reaction include benzaldehyde, acetaldehyde, acrolein, phenylglyoxal, and salicylaldehydeUnlike conventional Lewis acids, the lanthanide triflates are not destroyed in workup and can be recovered and reused. These reactions are especially useful when using small aldehydes, such as formaldehyde and acetaldehyde, which are commercially available as aqueous solutions.

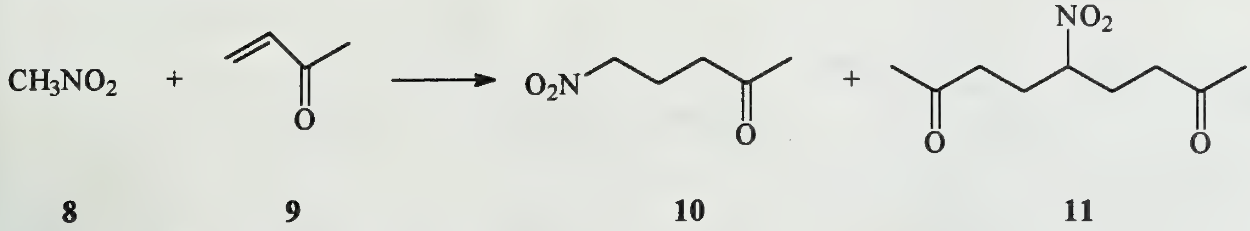


5 **6** **7**

Scheme II. The Reformatsky reaction.

The Reformatsky reaction (scheme II) is another aldol. In this reaction, an α-bromo ketone (e.g **5**) is added to an aldehyde (e.g. **6**) in the presence of a metal to produce the product. This reaction has been performed in water with no evidence of any self-condensation or other side reactions typical of aldol condensations.[10] It proceeds by single electron transfer to the metal to generate an α-keto radical, instead of through the classical enolate anion. In water, an interesting selectivity was observed. Aliphatic α-bromo ketones reacted only with aromatic aldehydes, and not with aliphatic aldehydes, while α-bromo aryl ketones could react with both kinds of aldehydes. Presumable an aromatic ring is necessary to add stability to the radical intermediate.

THE MICHAEL REACTION



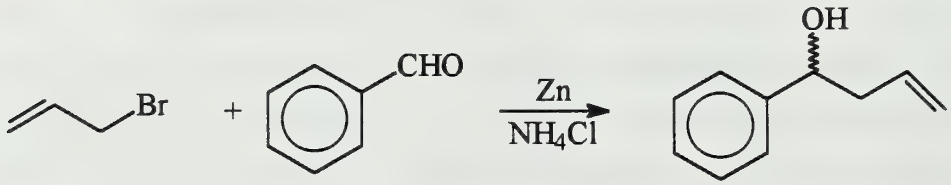
Scheme III. The Michael reaction of nitromethane with methylvinylketone.

The Michael reaction is also typically performed in an organic solvent with basic catalysis. Michael reactions are accelerated under pressure,[11] so they might be subject to hydrophobic acceleration. The reaction of nitromethane **8** with methyl vinyl ketone **9** (Scheme III) occurred in neutral aqueous solution.[12] The reaction was also possible without catalyst in methanol and dimethylsulfoxide, although it took longer, and more of bis adduct **11** was isolated. This is attributed to the lower solubility of mono adduct **10** in water than organic solvents. The authors propose a hydrophobic interaction as the cause of the rate acceleration in water. They also obtained quantitative yields of mono-addition product using nitroethane and methyl vinyl ketone in water.

Luche and co-workers have developed conjugate additions to unsaturated carbonyl compounds in aqueous solution using alkyl iodides.[13] The reaction proceeds through the free radical intermediate. Alkyl halides and unsaturated carbonyl compounds were treated with zinc powder and CuI and subject to ultrasonic irradiation. The reactivity increased with increasing substitution on the alkyl halide. This reaction provides a acceptable alternative to conventional organometallic methods of conjugate addition, such as the Gilman reaction. The reaction was tested with unrestricted, cisoid, and transoid ketones and aldehydews. The reaction shows a wide tolerance of steric and electronic effects in the carbonyl compound.

THE BARBIER REACTION

The Barbier reaction,[14] (scheme IV) is one of the most important methods available for the preparation of homoallylic alcohols. It involves the condensation of an allyl halide with a ketone or aldehyde in the presence of a metal.



12 **13** **14**

Scheme IV. The Barbier reaction.

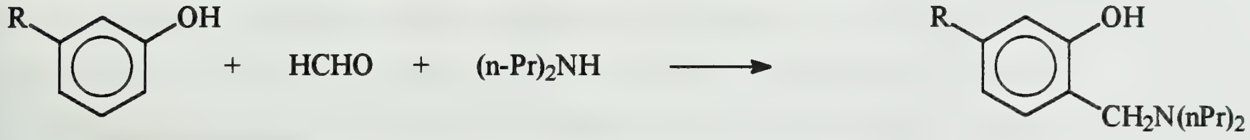
This reaction has been traditionally done in ethereal solvents and has been thought to involve the *in situ* generation of the allyl organometallic species. A radical mechanism was proposed for this reaction in 1982.[15] In 1983, Nokami discovered that the Barbier reaction could be done in the presence of water when it failed to work well under anhydrous conditions.[16] Since that time, aqueous media have been shown to be ideal for this reaction.

Luche developed the aqueous allylation of aldehydes and ketones in mixed water/THF solution using ultrasonic irradiation or NH_4Cl to activate the metal, usually zinc or tin.[17] The reactions typically are done in less than an hour and high yields are obtained. The compatibility of these reactions with water rules out the organometallic mechanism and suggests instead a radical mechanism. The aqueous reaction is compatible with several functional groups that often require protection during organometallic additions to carbonyl groups. The most noteworthy examples are hydroxyl groups and carboxylic acids. Whitesides has even allylated unprotected carbohydrates in aqueous ethanol.[18]

A study of the stereochemistry of the reaction of allyl bromide on several ketones[17b] revealed no differences between the aqueous and anhydrous variations of the Barbier reaction. In addition, chiral additives had no effect on the stereoselectivity of the reaction. The medium of the reaction is thus unimportant, and apparently plays no significant role in the mechanism. Nonetheless, the aqueous variant has several advantages. The reaction can tolerate functional groups that are usually incompatible with metal-catalyzed reactions, such as hydroxyl groups and carboxylic acids.

Furthermore, ketals are hydrolyzed and allylated when using tin, but zinc leaves the protecting group intact.

OTHER REACTIONS

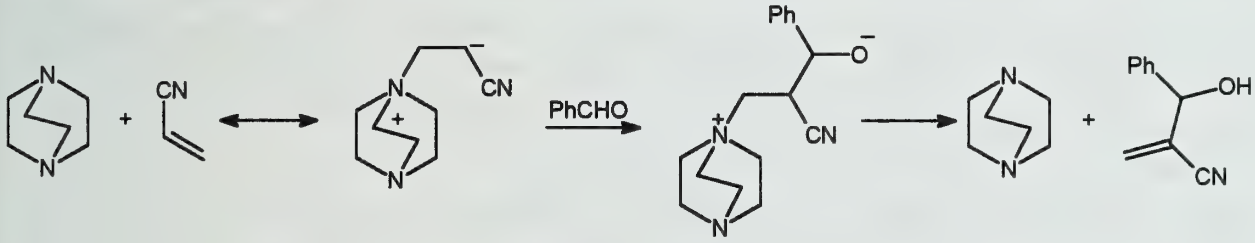


15a: R=H **16** **17** **18a:** R=H

15b: R=$(CH_2)_{15}CH_3$ **18b:** $CH_2)_{15}CH_3$

Scheme V. The Mannich reaction.

Other types of carbonyl addition occur in water. The Mannich reaction has shown rate accelerations in aqueous solutions (Scheme V). The reaction is very slow in ethanol, but procedes to completion in 2.5 hours in water.[19] The reactants were fully soluble in the ethanol, but formed a biphasic mixture in water. The large rate increase is most likely due to solvent polarity and hydrophobic effects. Kobayashi has recently shown that $Yb(OTf)_3$ acts as an effective Lewis acid catalyst in the Mannich reaction.[20] Under Lewis acid catalysis, the reaction proceeds smoothly with anilines and substituted aldehydes.



19 **20** **21** **22** **23**

Scheme VI. The Baylis-Hillman reaction.

The Baylis-Hillman reaction (Scheme VI) has also shown rate enhancement in aqueous solution.[21] In fact, the reaction is complete in 8 hours in water, compared to 34 hours in methanol. This reaction has a charged intermediate (**21**) that can be stabilized by water. Salting-out effects also indicate hydrophobic interactions are involved as part of the rate enhancement. Accelerations in water are also observed with other aldehydes, including acetaldehyde, butanal, and furaldehyde. The reaction in furaldehyde was especially rapid, taking place within 2 hours.

CONCLUSIONS

Many common organic reactions can occur in aqueous solution. Several reactions that have been traditionally thought of as being moisture sensitive occur in water with little or no modifications. Even some water-intolerant species have equivalent reactions that take place in aqueous solution. The use of water has many advantages, both in the ease of use and the effects on the chemistry taking place. More work needs to be done to examine the specific effects of water, in order to be able to take advantage of water's unique properties.

REFERENCES

1. a) Lubineau, A.; Augé, J.; Queneau, Y. *Synthesis.* **1994**, 741. b) Li, C.J. *Chem. Rev.* **1993**, *93*, 2023.
2. Ben-naim, Arieh. Hydrophobic Interactions. New York: Plenum Press, 1980.
3. Breslow. R. *Acct. Chem. Res.* **1991,** *24,* 159.
4. Fringuelli, F.; Pani, G.; Piermatti, O.; Pizzo, F. *Tetrahedron.* **1994**, *50*, 11499.
5. Buonora, P.T.; Rosauer, K.G.; Dai, L. *Tetrahedron Lett.* **1995,** *36,* 4009.
6. Mukaiyama, T.; Banno, K.; Narasaka, K. *J. Am. Chem. Soc.* **1974**, *96*, 7503.
7. a) Lubineau, A. *J. Org. Chem.* **1986,** *51,* 2144. b) Lubineau, A.; Meyer, E. *Tetrahedron* **1988,** *44,* 6065.
8. Yamamoto, Y.; Maruyama, K.; Matsumoto, K. *J. Am. Chem. Soc.* **1983,** *105*, 6963.
9. a) Kobayashi, S. *Synlett* **1994,** 1994. b) Kobayashi, S.; Hachiya, I. *J. Org. Chem.* **1994**, *59,* 3590. c) Kobayashi, S.; Hachiya, I.; Yamanoi, Y. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 1994.
10. Chan, T.H.; Li, C.J.; Wei, Z.Y. *J. Chem. Soc., Chem. Comm.* **1990,** 505.
11. Matsumoto, K. *Angew. Chem. Int. Ed. Engl.* **1981,** *20*, 770.
12. Lubineau, A.; Augé, J. *Tetrhedron Lett.* **1992,** *33,* 8073.
13. Luche, J.L.; Petrier, C.; Dupuy, C. *Tetrahedron Lett.* **1986,** *27,* 3149.
14. Barbier, P.L. *Cr. R. Hebd. Seances Acad. Sci.* **1898,** *128,* 110.
15. Bauer, P.; Molle, G. *J. Am. Chem. Soc.* **1982,** *104,* 3481.
16. Nokami, J.; Otera, J.; Sudo, T.; Okawara R. *Organometallics* **1983,** *2,* 191.
17. a) Luche, J.L.; Pétrier, C. *J. Org. Chem.* **1985,** *50,* 910. b) Luche. J.L.; Einhorn, C. *J. Organomet. Chem.* **1987,** *322,* 177.
18. Schmid, W.; Whitesides, G.M. *J. Am. Chem. Soc.* **1991,** *113,* 6674.
19. Tyman, J.H.P.; Tychopoulos, V. *Synth. Comm.* **1986,** *16,* 1401.
20. Kobayashi, S.; Ishtani, H. *J. Chem. Soc., Chem. Comm.* **1995,** 1379.
21. Augé, J.; Lubin, N.; Lubineau, A. *Tetrahedron Lett.* **1994,** *35*, 7947.

LIGATION STRATEGIES IN PROTEIN SYNTHESIS

Reported by Lisa Flugge April 11, 1996

INTRODUCTION AND BACKGROUND

Chemical synthesis has become an increasingly popular means of assembling proteins.[1] Although molecular biology techniques enable expression and purification of proteins, there are advantages to synthesizing proteins chemically. For example, chemical synthesis allows introduction of non-natural and isotopically labeled amino acids into the protein for structure-function and spectroscopic studies. It also allows for the assembly of proteins that are difficult to express.

Initially, all protein synthesis was done in solution, which by all accounts was an arduous task. The synthesis of longer peptides and proteins did not become feasible until 1963, when Merrifield developed solid phase peptide synthesis (SPPS).[2] This development won Merrifield the Nobel Prize in 1984. SPPS has been greatly refined since and the standard methods used in solid phase synthesis today are the Boc (t-butoxycarbonyl) or Fmoc (9-Fluorenylmethoxycarbonyl) strategies.[1] These refer to the N-terminal protecting group used and differ in the deprotection strategy. (Figure 1) Boc chemistry employs gradient based protection of side chains and the N- terminus while Fmoc uses an orthogonal scheme.

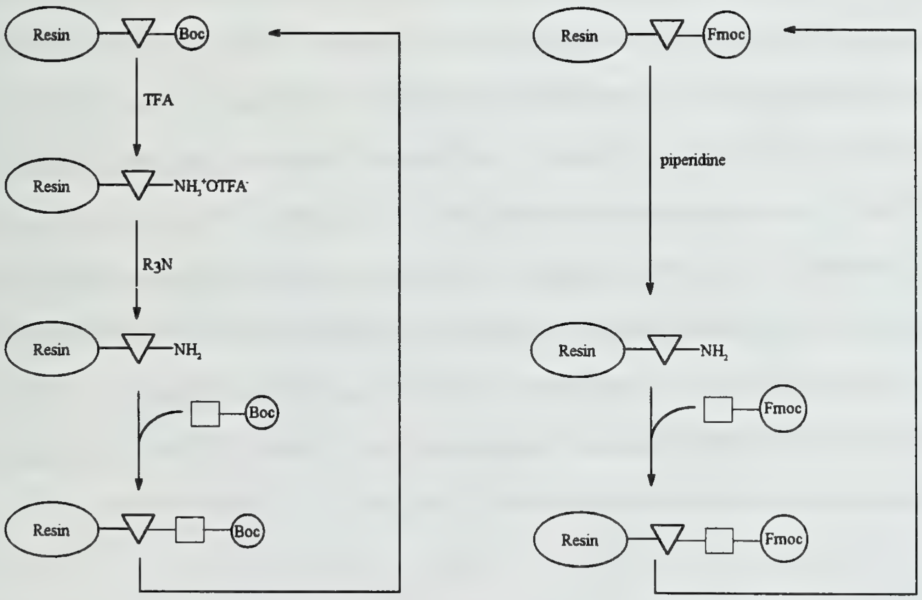


Figure 1. Boc and Fmoc strategies. TFA=trifluoracetic acid

Copyright © 1996 by Lisa Flugge

By attaching the C-terminus to a resin and differentially protecting the side chains and N-terminus, it is possible to build up a protein stepwise on the resin. Cleavage from the resin and deprotection of the side chains result in the final product. (Figure 1) As SPPS has become more refined, larger and larger structures have become feasible. Several proteins have been made by this method including RNase A, Interleukin-8, and D-HIV protease. [3] To date, the largest proteins feasible by solid phase synthesis are about 100 amino acids in length.

Several problems exist when synthesizing a complete protein on the solid phase resin. Many of the protected amino acids are not very soluble in the solvents used for coupling. Longer chains may begin to associate with each other. In addition, as protein chains get longer, more side products corresponding to deletions in the sequence accumulate.[1] These side products make purification of the resulting protein very difficult. Proteins of 100 amino acids or more can be synthesized by solid phase techniques, but require special care in preparation and purification. Chains of around 30 amino acids can be routinely made and purified. A way to circumvent the problem of creating longer peptide chains would be to synthesize smaller peptide fragments and stitch them together. There are currently several ways of ligating peptides involving both chemical and enzymatic means.

CONDENSATION

One of the simplest ways to ligate peptides would be to synthesize smaller fragments and bring these fragments together on the solid support, an approach known as condensation.[4] Although conceptually simple, condensation has several disadvantages. The larger protected fragments are poorly soluble under the reaction conditions, and therefore difficult to react. Kaiser and coworkers circumvented this problem through the segment synthesis condensation approach.[5] This method involves creating the smaller protected fragments on a solid support and then bringing them together in solution. Several groups have demonstrated the usefulness of this method through the synthesis of proteins such as apolipoprotein A1, ribonuclease T_1,[5] and amyloid βA4,[6] which had eluded traditional solid phase techniques. The condensation and segment condensation approaches both require full side chain protection, which can cause problems with solubility and yield.

NON-AMIDE LINKAGES

There are several ways to link peptide fragments together that do not employ amide bonds.

Having a non-amide bond may be useful when looking at the role of specific hydrogen bonds to the protein backbone. In addition, non-amide linkages open up different kinds of compatible reactions. One non-amide bond utilizes a hydrazone linkage.[7] Hydrazone linkages had been previously used in covalent modification of proteins,[8] and involve reaction of an N-terminal aldehyde from threonine or serine with hydrazide under mild conditions. (Figure 2)

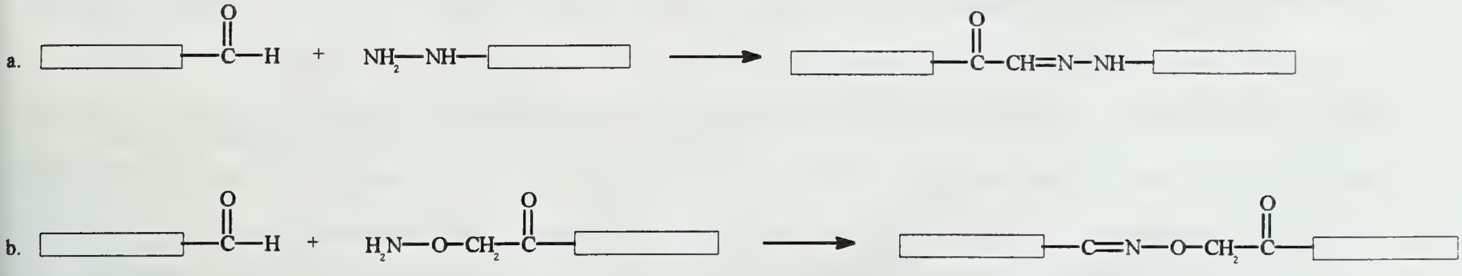


Figure 2. Preparation of a. hydrazone and b. oxime linkages

The aldehyde can be made by oxidizing a protein with periodate which selectively reacts with N-terminal secondary alcohols to give a glyoxal functionality. Hydrazides can be made on the solid support. Since this is a specific reaction, hydrazone formation does not require use of side chain protecting groups, eliminating some of the solubility problems faced in condensation. However, hydrazone linkages tend to undergo hydrolysis under both acidic and basic conditions. The hydrazone linkage in covalent modification of proteins was originally designed to allow for slow hydrolysis and release of the conjugate, which is a disadvantage in protein synthesis. Hydrazone has been used to ligate both human granulocyte colony stimulating factor[7] and peptide dendrimers.[9]

A similar linkage is based on oxime,[10] which like hydrazone, has been used in covalent modification of proteins.[11] Oxime linkages are formed by selectively reacting a terminal aldehyde made from an N-terminal serine or threonine with an aminooxyacetyl group. This can be done in the absence of side chain protection, again circumventing solubility problems. Oxime linkages have been applied to peptide dendrimers and to link two domains of the cMyc-Max heterodimer.[12] A significant drawback of oxime linkages is that they are unstable at basic pH. Also, secondary structure is an important factor to consider in both cases. Oxime and hydrazone linkages do not have the same geometry as amide links so they can disrupt secondary or tertiary structure of highly defined regions.

Sulfur chemistry offers a large assortment of potential linkages for protein synthesis. Cysteine, a sulfur containing residue, is present in most proteins. Kent and co-workers have extensively studied the thioether[13] and thioester linkages.[14] Thioethers are made by selectively reacting the sulfhydryl group of a terminal cysteine with a terminal bromoacetyl group.(Figure 3a) The pH of this reaction must be carefully controlled to ionize the sulfhydryl group without affecting any other side chains. In addition, no other cysteine residue can be available in the molecule for reaction. In the presence of other cysteine residues the reaction will not be selective and side reactions will occur. Solvent conditions must also be controlled to prevent the formation of the disulfide.

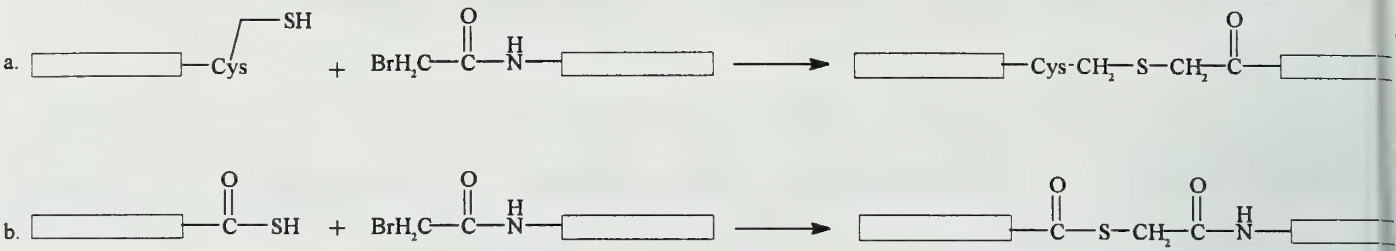


Figure 3. a. Thioether formation b. Thioester formation

Kent has also investigated thioester linkages for the synthesis of HIV-1 protease.[14] The thioester linkage has been used in a number of other proteins, such as a four helix template assembled synthetic protein and a domain of fibronectin.[15] The thioester linkage is the closest isoster to the normal peptide linkage, and also eliminates a hydrogen bond donor and acceptor. To selectively form the thioester, a terminal thioacid is reacted with a terminal bromoacetyl. (Figure 3b) The thioacid and bromoacetyl groups are made on the solid support during SPPS. The reaction is usually run in 6M guanidine chloride to ensure the protein is fully denatured, which avoids any steric interference that could be caused by the secondary structure of the protein. Unlike other linkages based on sulfur chemistry, thioester ligation works in the presence of unprotected cysteine residues. Kent postulates that the ligation must occur at a glycine residue in order for the SN2 displacement of bromine to proceed at a reasonable rate. In addition, the thioester bond has the disadvantage of lability at neutral and basic pH. Kent found complete hydrolysis of his HIV protease in solution of pH 7.5 after 2 hours. These problems prevent the thioester linkage from being universally applied.

AMIDE LINKAGES

Naturally, amide links in engineered proteins would be ideal, and considerable progress has been made in creating these linkages as well. Autocatalytic fragment religation (AFR) is one of the first methods of amide bond formation investigated.[16] Development of this method stemmed from the observation that observation that occasionally protein segments obtained by digesting the protein with a specific cleavage agent will reassociate non-covalently in solution and have the same characteristics as the folded, intact protein. In some cases these proteins will actually religate (Figure 5). Placing a reactive group at the carboxy terminus will ensure the protein fragments will re-ligate in solution. The reactive group can either be a result of the method used to cleave the protein originally or be placed there chemically. For instance, cleavage of cytochrome *c* with CNBr will give a reactive homoserine lactone at the C-terminus. The C-terminus can also be selectively activated, usually as the chlorophenyl ester. There are several limitations to AFR. In order for AFR to work, the segments being ligated have to fold into their eventual three dimensional structure and associate with each other. In addition, cleavage agents such as cyanogen bromide cut at specific places in the molecule, so the primary structure needs to be considered. Finally, the secondary structure of the protein must be considered. For example, the religation does not work very well in hydrophobic areas of the protein or in sharp β-turns. AFR has been used most extensively in semisynthesis, where a man-made fragment is ligated to a fragment from the native protein. This approach has been used in the engineering of cytochrome *c*[17] and other proteins.[18]

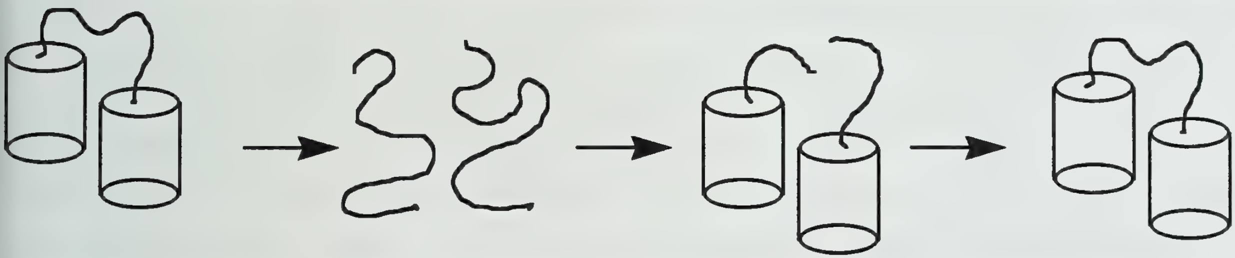


Figure 6. Autocatalytic Fragment Religation

Tam and coworkers have looked at thiazolidine linkages.[9,19] This ligation introduces a proline-like residue into the backbone of the protein. It is accomplished by reacting a C-terminal glycoaldehyde, usually glycine or alanine, with an N-terminal cysteine. (Figure 7) The N-terminal aldehyde can either be synthesized chemically or enzymatically. Tam has utilized the thiazolidine linkage in model studies with short peptides and in peptide dendrimers. Care must be taken in selecting a site for ligation, as proline residues are very disruptive to secondary structure. The proline-like thiazolidine will have similar effects on the protein. The thiazolidine linkage is stable under a large range of pH and the selective reaction occurs fairly quickly. Tam is currently studying ways to open up the thiazolidine ring and form a free cysteine, but currently the thiazolidine linkage is best regarded as a substitute for proline.

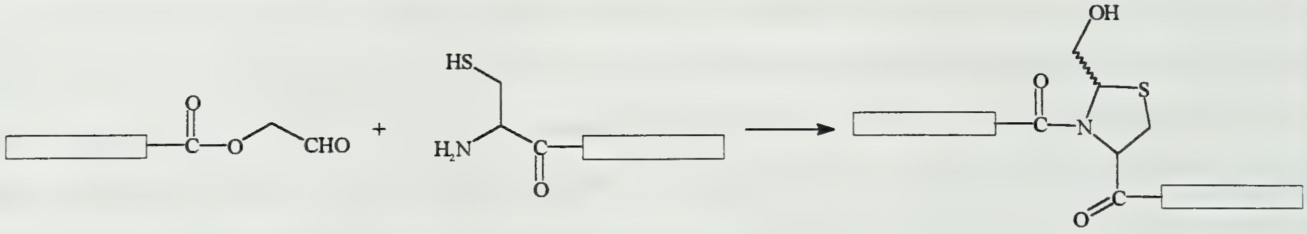


Figure 7. Thiazolidine formation.

Kemp and coworkers have investigated thiol capture reactions to form peptide bonds.[20] His strategy utilizes a template that increases effective molarity and induced proximity effects in the reacting peptides. (Figure 8)

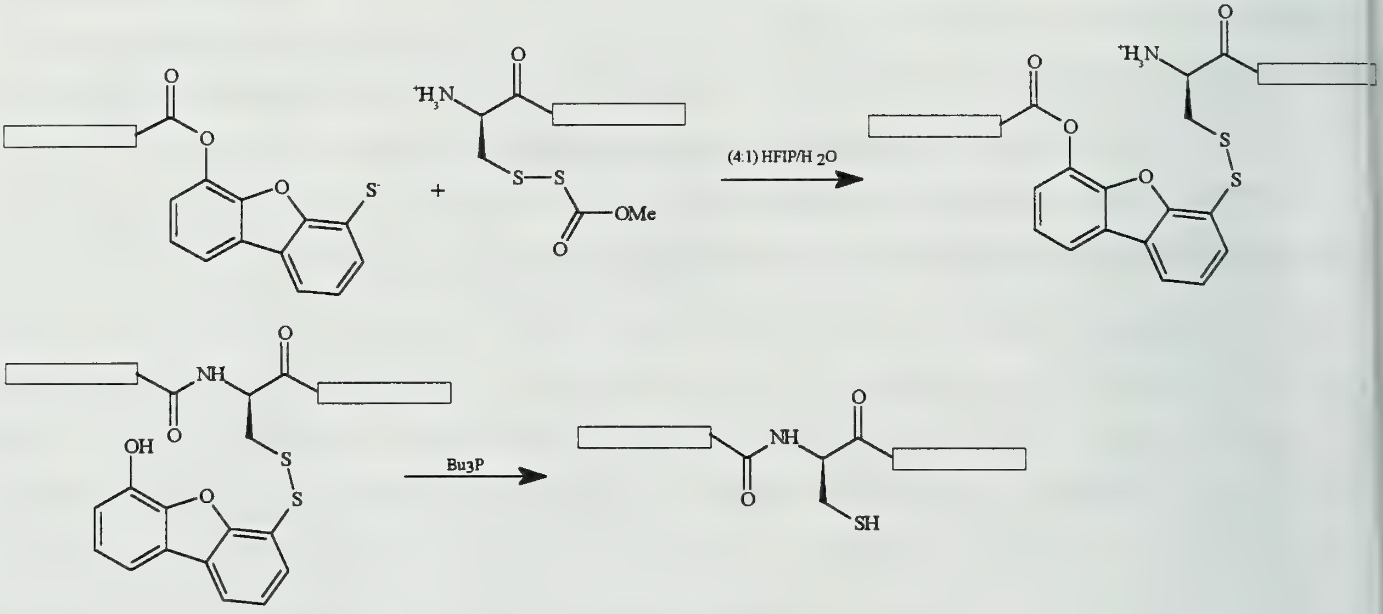


Figure 8. Thiol Capture

Here again a cysteine residue is needed at the ligation site. A disulfide is formed by the cysteine with the template molecule. By deprotonating the amine, the amide forming reaction occurs. The template is removed by reducing the disulfide with Bu$_3$P. This method requires the protection of cysteine residues that are not involved in the reaction, but no other side chains require blocking.

ENZYME-ASSISTED LIGATION

Enzymes have been developed that are powerful tools in the field of protein ligation. The most widely used enzyme is subtiligase, a mutant of the serine protease subtilisin.[21] Using a protease to form amide bonds in proteins may seem counterproductive, but by changing the enzyme through selective mutations and by adjusting reaction conditions, the enzyme can be made to favor aminolysis over hydrolysis. Wild type subtilisin hydrolyzes amides and esters.[22] The enzyme binds an extended conformation of the protein to be cleaved, four residues on the N-terminal side (P1-P4) and three residues on the C-terminal side (P1'-P3'). The serine in the active site works to form an acyl enzyme intermediate. This intermediate is subsequently hydrolyzed.

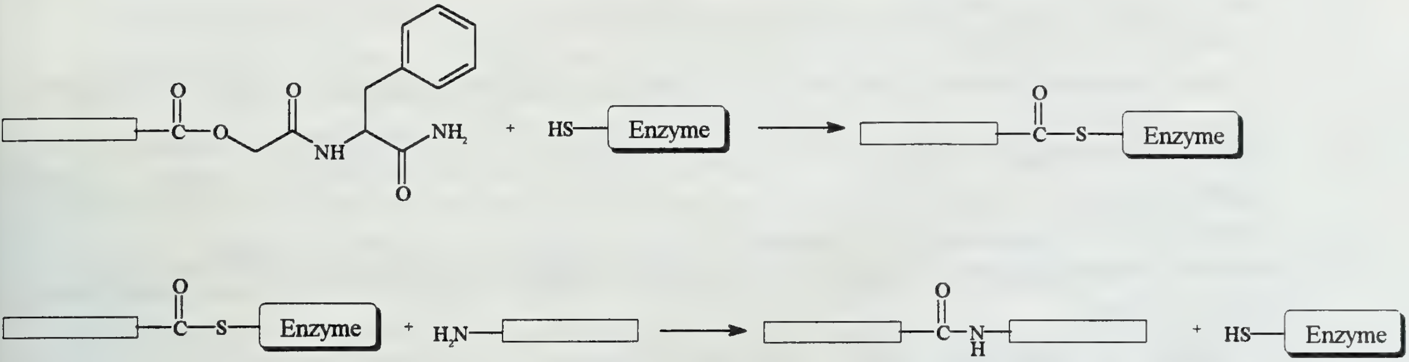


Figure 9. Peptide ligation using subtiligase

Thiosubtilisin is a subtilisin mutant with the active site serine mutated to cysteine (S221C).[22] This mutant enzyme has been used to study the activity and mechanism of subtilisin. Gray and co-workers surmised that the hindered catalytic activity might lend itself to ligating peptides. When they tested thiosubtilisin for ligating activity, they found it ligated at a very reduced rate.[23] Wells et. al. converted an active site proline to an alanine in thiosubtilisin. This mutation reduced active site crowding and increased the enzyme's ligation activity. They dubbed this mutant subtiligase.[24] Subtiligase works in a similar manner to subtilisin, with the active site cysteine forming a thioester-enzyme intermediate which subsequently undergoes aminolysis. (Figure 9) Since subtiligase is specific for certain kinds of residues, ligations need to be made at sites compatible with these preferences. In addition, the ligation will not work well in regions of highly defined secondary structure such as an α-helix, as the enzyme binds an extended conformation of the protein to be ligated. Subtiligase has been applied to the synthesis of RNase A and cyclic peptides.[24]

CONCLUSION

In conclusion, there are many different methods available for ligating peptides in protein synthesis. Clearly, no one method is best, as each have different requirements for primary, secondary, and tertiary structure, as well as differing stabilities. A distinct problem with the field is that the scope of each ligation method is not clear and should be defined. In the future, work needs to be done on determining a general method for protein ligation. Currently the most promising method appears to be subtiligase.

REFERENCES

1) a) Kent, S.B.H. *Ann. Rev. Biochem.* **1988**, *57*, 957. b) Kaiser, E.T. *Acc. Chem. Res.* **1989**, *22*, 47. c) Hilvert, D. *Chemistry and Biology* **1994**, *1*, 201.
2) Merrifield, R.B. *J. Am. Chem. Soc.* **1963**, *85*, 2149.
3) a)Milton, R.C.D.; Milton, S.C.F.; Kent, S.B.H. *Science* **1992**, *256*, 1445. b) Clark-Lewis, I.; Moser, B.; Walz, A.; Baggiolini, M.; Scott, G.J.; Aebersold, R. *Biochemistry* **1991**, *30*, 3128.
4) Denkewalter, R.G.; Veber, D.F.; Holly, F.W.; Hirschmann, R. *J. Am. Chem. Soc.* **1969**, *91*, 502.
5) Kaiser, E.T.; Mihara, H.; Laforet, G.A.; Kelly, J.W.; Walters, L.; Findeis, M.A.; Sasaki, T. *Science* **1989**, *243*, 187.
6) Hendrix, J.C.; Halverson, K.J.; Lansbury, P.T. *J. Am. Chem. Soc.* **1992**, *114*, 7930.
7) Gaertner, H.F.; Rose, K.; Cotton, R.; Timms, D.; Camble, R.; Offord, R.E. *Bioconjugate Chem.* **1992**, *3*, 262.
8) a) King, T.P.; Zhao, S.W.; Lam, T. *Biochemistry* **1986**, *25*, 5774. b) Geoghegan, K.F.; Stroh, J.G. *Bioconjugate Chem.* **1992**, *3*, 138.
9) Shao, J.; Tam, J.P. *J. Am. Chem. Soc.* **1995**, *117*, 3893.
10) Rose, K. *J. Am Chem Soc.* **1994**, *116*, 30.
11) Kurth, M.; Pelegrin, A.; Rose, K.; Offord, R.E.; Pochonis, S.; Mach, J-P.; Buchegger, F. *J. Med. Chem.* **1993**, *36*, 1255.
12) Canne, L.E.; Amare, A.R., Burley, S.K.; Kent, S.B.H. *J. Am. Chem. Soc.* **1995**, *117,* 2998.
13) Muir, T.W.; Williams, M.J.; Ginsberg, M.H.; Kent, S.B.H. *Biochemistry* **1994**, *33*, 7701.
14) Schnölzer, M.; Kent, S.B.H. *Science* **1992**, *256*, 221.
15) a) Baca, M.; Kent, S.B.H. *Proc. Natl. Acad. Sci.* U.S.A. **1993**, *90*, 11638. b) Dawson, P.E.; Kent, S.B.H. *J. Am. Chem. Soc.* **1993**, *115*, 7263.
16) a) Rose, K.; Herrero, C.; Proudfoot, A.E.I.; Offord, R.E.; Wallace, C.J.A. *Biochem. J.* **1988**, *249*, 83. b) Proudfoot, A.E.I.; Rose, K.; Wallace, C.J.A. *J. Biol. Chem.* **1989**, *264*, 8764.
17) a) Wallace, C.J.A.; Guillemette, J.G.; Hibriya, Y.; Smith, M. *J. Biol. Chem.* **1991**, *266*, 21355. b) Wallace, C.J.A. *FASEB J.* **1993**, *7*, 505. c) Wuttke, D.S.; Gray, H.B.; Fisher, S.L.; Imperiali, B. *J. Am. Chem. Soc.* **1993**, *115*, 8455.
18) Dawson, P.E.; Muir, T.W.; Clark-Lewis, I.; Kent, S.B.H. *Science* **1994**, *266*, 776.
19) a) Liu, C-F.; Tam, J.P. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91,* 6584. b) Liu, C-F.; Tam, J.P. *J. Am. Chem. Soc.* **1994**, *116*, 4149.
20) Kemp, D.S.; Carey, R.I. *J. Org. Chem.* **1993**, *58*, 2216.
21) Abrahmsen, L.; Tom, J.; Burnier, J.; Butcher, K.A.; Kossiakoff, A.; Wells, J.A. *Biochemistry* **1991**, *30*, 4151.
22) a) Philipp, M.; Bender, M.L. *Mol. Cell. Bioch.* **1983**, *51*, 5. b) Estell, D.A.; Grayear, T.P.; Miller, J.V.; Powers, D.B.; Burnier, J.P.; Ng, P.G.; Wells, J.A. *Science* **1986**, *233*, 659.
23) a) Nakatsuka, T.; Sasaki, T.; Kaiser, E.T. *J. Am. Chem. Soc.* **1987**, *109*, 3808 b) Jackson, D.Y.; Burnier, J.; Quan, C.; Stanley, M.; Tom, J.; Wells, J.A. *Science* **1994**, *266*, 243. c) Chang, T.K.; Jackson, D.Y.; Burnier, J.P.; Wells, J.A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 12544.
24) Jackson, D.Y.; Burnier, J.P.; Wells, J.A. *J. Am. Chem. Soc.* **1995**, *117*, 819.

EVOLUTION'S MIDDLE-AGE: CHEMICAL PROGENITORS OF LIFE

Reported by Brian Hubbard April 25, 1996

INTRODUCTION AND BACKGROUND

For centuries, scientists have debated the issues of evolution and the origins of life. Darwin's theory of evolution, termed "natural selection," explains evolutionary trends based upon observable phenotypes arising from genetic mutations. Evolution through natural selection has driven the rise and fall of life from its origins. Since the original Urey-Miller experiment in 1952, in which a spark discharge in an atmosphere of H_2O, CH_4, NH_3 and H_2 gave rise to many common building-blocks of today's biological systems, scientist have been struggling to develop a viable chemical model for prebiotic evolution. To date, no chemical theory has been able to experimentally produce a fully competent, self-assembled, self-replicating system from the small inorganic molecules believed to be available on primitive earth.

The first information storage and transfer system is proposed to have arisen in one of three ways. In the "Protein World," proteins are believed to be the information transfer and storage material which evolved and subsequently gave rise to RNA. The "RNA World" proposes that RNA arose prior to protein catalysts. Some RNA World advocates also believe in a "Pre-RNA World" in which molecules similar to RNA, but consisting of more accessible monomer units, provided a template for RNA evolution. All of these theories require the efficient prebiotic synthesis of organic precursor molecules such as amino acids, pyrimidines, purines and sugars. The focus of this abstract is on how small inorganic molecules combine in a kinetically competent, mechanistically sound manner to yield the basic building blocks of life.

The development of the chemical progenitors of "life" was certainly dependent upon the environment on primitive Earth. There is wide disagreement about the exact nature of this environment, which primarily depends upon whether iron in the earth's crust had settled to the core. If iron had not settled to the core, the atmosphere would have been strongly reducing, consisting primarily of H_2, H_2O, NH_3, CH_4 and CO. If iron had settled to the Earth's core,

Copyright © 1996 by Brian Hubbard

which is the current scientific dogma, the atmosphere would predominantly consist of H_2O, CO_2, H_2,and CO with little NH_3 or CH_4 present. Even if CH_4 and NH_3 were present on early earth they would have likely undergone photodecomposition.[1]

PROTEIN WORLD

The major challenge in developing a viable protein under prebiotic conditions is the synthesis and coupling of amino acid subunits. [2,3,4] Diaminomaleonitrile (DAMN) can be readily formed under mildly reducing conditions[5] via HCN oligomerization (Figure 1). Further oligomerization with HCN followed by hydrolysis, yields mixtures predominantly composed of glycine, alanine, aspartate, and diaminosuccinate.[6]

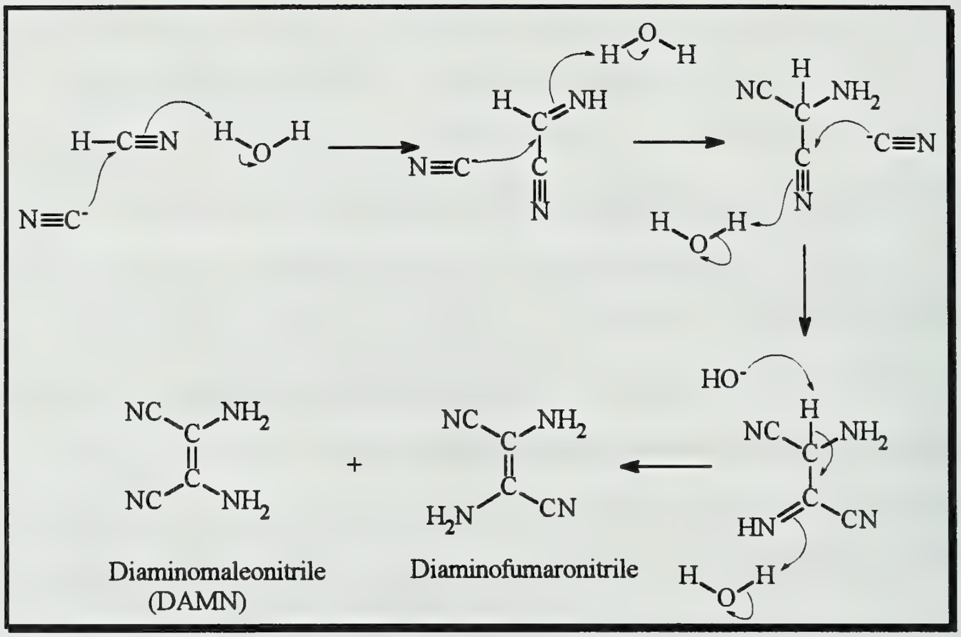


Figure 1: Synthesis of diaminomaleonitrile via HCN oligomerization

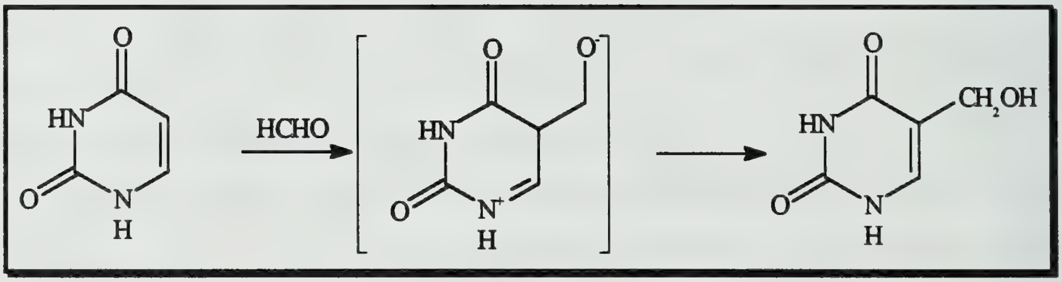


Figure 2: 5-hydroyxmethyluracil synthesis

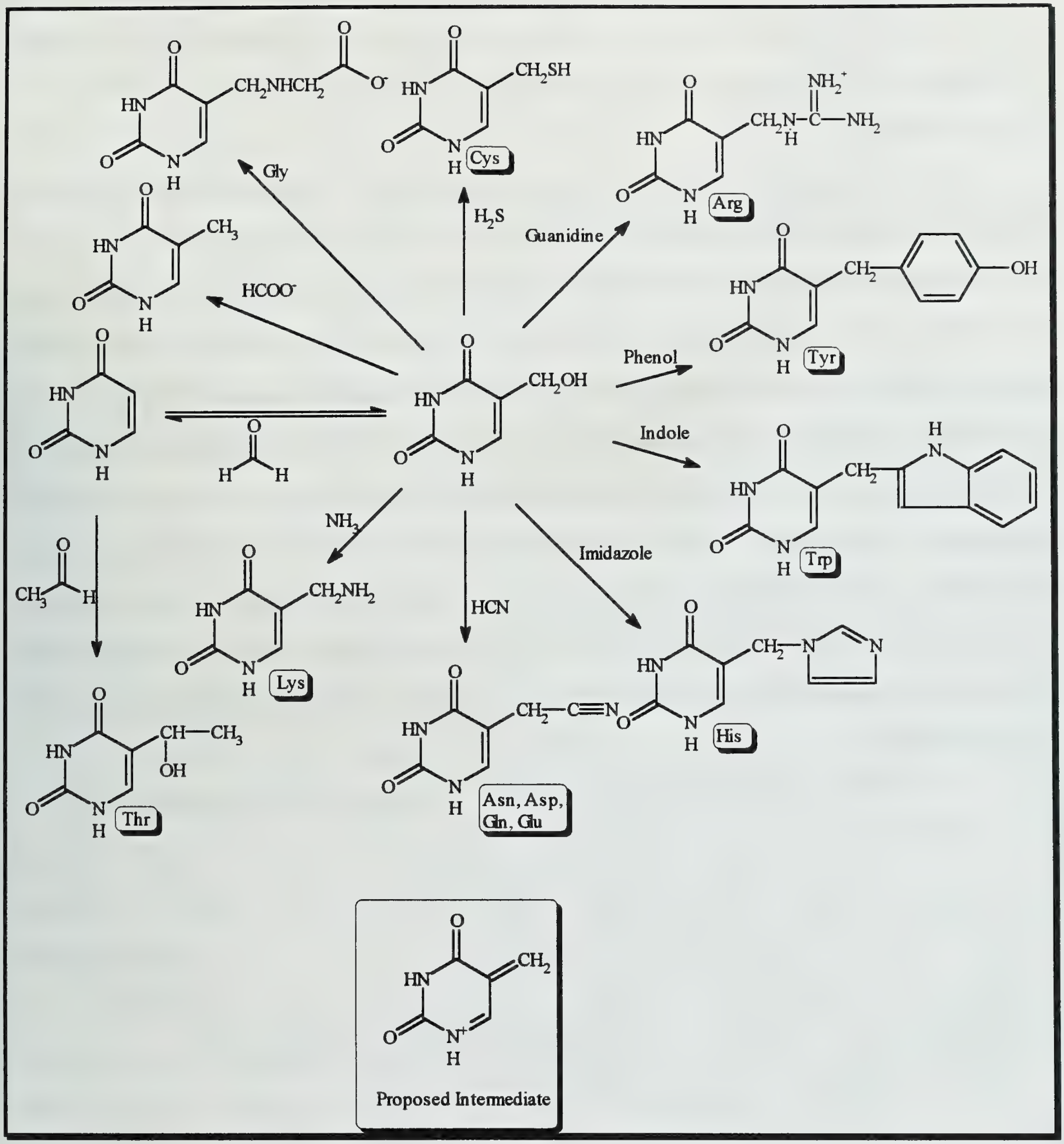


Figure 3: Amino Acid Analogues

Miller has identified 5-hydroxymethyluracil as a template onto which many of the common amino acid side-chains can be attached.[7] This template is the condensation product of uracil and formaldehyde (Figure 2). Dehydration results in a Michael acceptor capable of trapping a number of nucleophiles which are peptide sidechain equivalents or analogues (Figure 3). Sixteen of the twenty naturally occurring amino acids have a methylene group between the side chain and the amide backbone. In Miller's synthesis of amino acid analogues via 5-hydroxymethyluracil, there is a methylene group separating the amino acid sidechain from the uracil backbone.

RNA WORLD

For RNA to be the primordial means of information storage and transfer, purines, pyrimidines, ribose, and inorganic phosphates must have been readily available. Furthermore, these building blocks had to combine to yield a competent RNA molecule. Orgel showed that the purine bases adenine, diaminopurine, hypoxanthine, guanine and xanthine could be made from HCN and water.[8] He later demonstrated that the purines could be easily synthesized by self-condensation of HCN under a wide variety of conditions (Figure 4). In Orgel's first synthesis, he demonstrated that diaminomaleonitrile irradiated with UV light (350nm) yielded an imidazole intermediate. An alternative route to the same intermediate combines diaminomaleonitrile and formamidine. This intermediate, when combined with HCN or CH_2N_2 yields adenine or diaminopurine respectively. Hydrolysis of the imidazole intermediate gave the formamide. The formamide, when combined with HCN, CH_2N_2 or NCO^-, yielded hypoxanthine, guanine, or xanthine respectively.

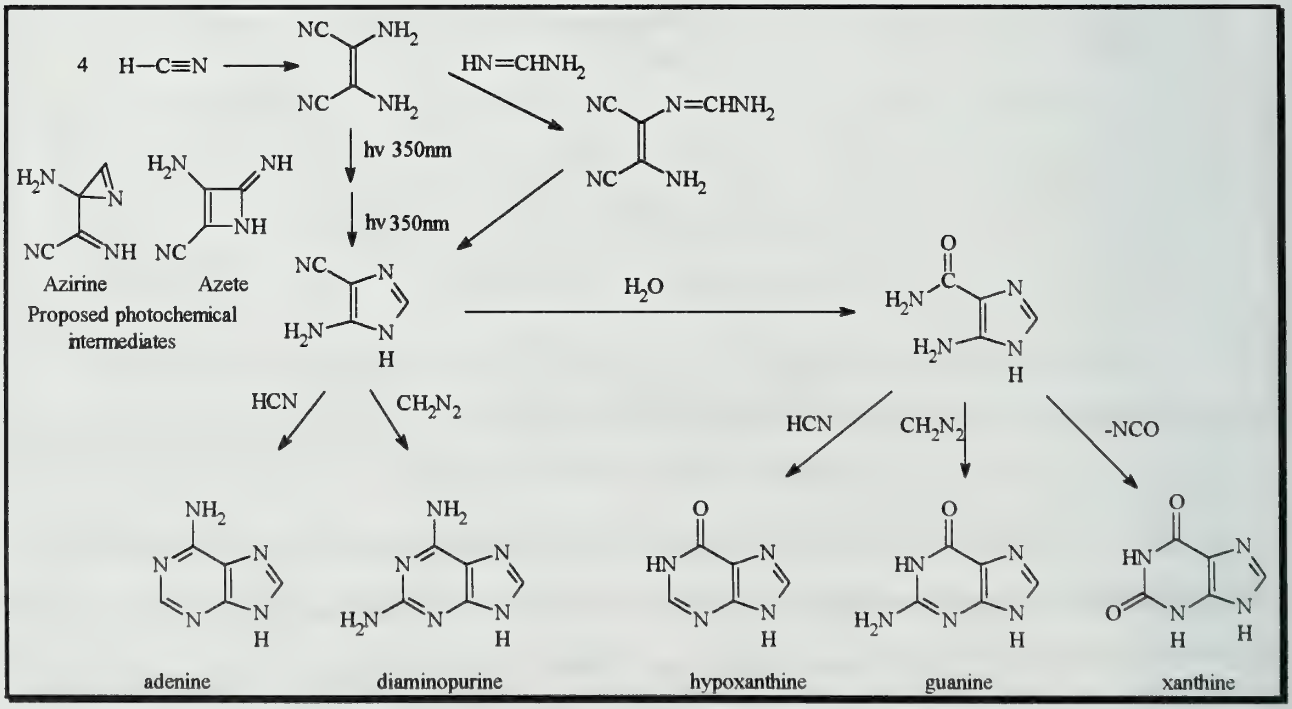


Figure 4: HCN synthesis of Purines

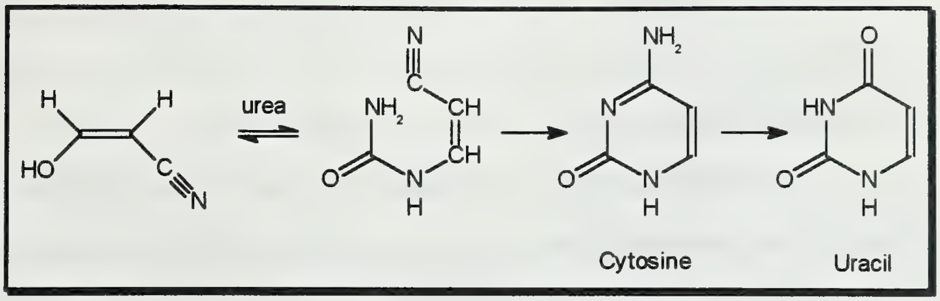


Figure 5: Prebiotic synthesis of cytosine and uracil

Controversy surrounds the synthesis of pyrimidines under prebiotic conditions. Numerous routes have been postulated for the synthesis of cytosine and uracil, but they are typically plagued by poor yields. For example, potassium cyanate (0.1M) and cyanoacetylene (0.1M) can combine to yield cytosine, but only in 5% yield.[9] Alternatively, the hydrolysis of HCN oligomers was shown to produce 4,5-dihydroxypyrimidine, 5-hydroxymethyluracil, and orotic acid. The latter underwent photochemical decarboxylation to yield uracil.[10] Miller has proposed a more efficient synthesis of cytosine and uracil from urea and 5-hydroxyacrylonitrile. Cytosine and uracil are produced in yields exceeding 50% (Figure 5). [11] However, Shapiro has been suggested that all of these methods for pyrimidine synthesis require concentrations of reactants too high to exist under prebiotic conditions.[12]

Formaldehyde has been proposed as the precursor for ribose under prebiotic conditions. When formaldehyde is stirred in an alkali solution, a series of aldol condensations and tautomerizations yields a mixture of >65 sugars. The formation of sugars by this process is termed the Formose or Butlerow reaction (Figure 6).[13] Gas

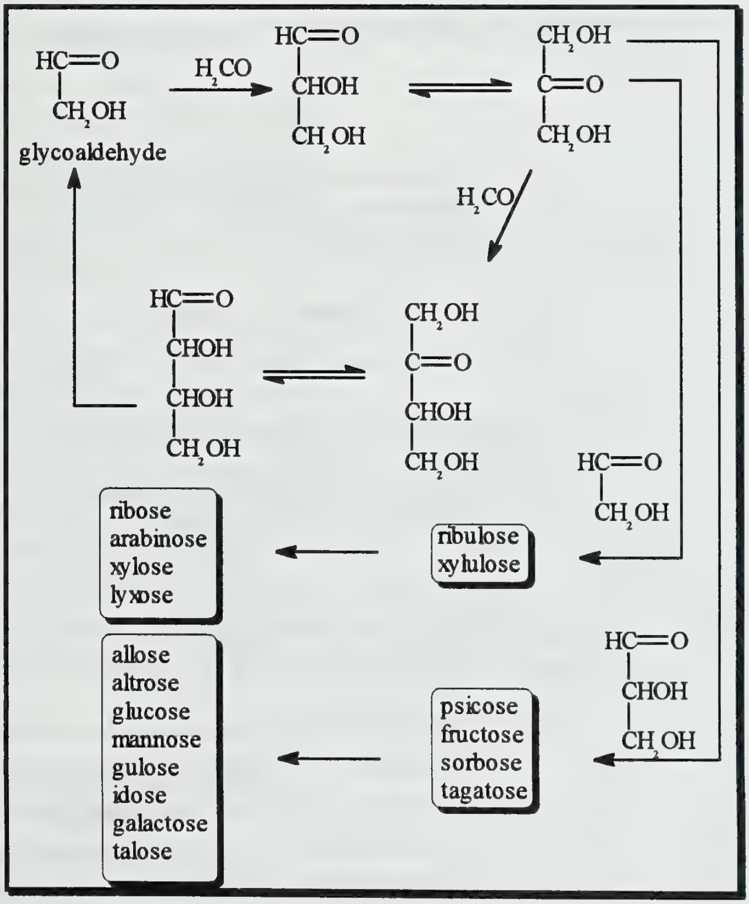


Figure 6: Formose (Butlerow) reaction

chromatography analysis of the formose reaction mixture indicates that there is no preference for ribose.

PRE-RNA World

The major shortcoming with the RNA world is the inability to selectively make ribose by the Butlerow reaction. Many original supporters of the “RNA World” now believe there was a “Pre-RNA World” in which molecules similar to RNA but consisting of different backbones served as the primitive information transfer and storage system (Figure 7). The glycerol backbone (Figure 6-B) has been the most widely studied because glycerol is one of the major products from the formose reaction. The guanine-glycerol hexamer exhibited mild templating characteristics in the presence of cytosine-glycerol monomers.[14]

Polyamide nucleic acids (PNA: 3’-amino-3’-deoxynucleiotides) have been shown to be efficient templates for PNA oligomerization.[15] Electron micrograph studies show that PNA can displace DNA and RNA dimers to form the PNA hybrid dimer.[16] However, the synthesis of PNA under prebiotic conditions has yet to be demonstrated.

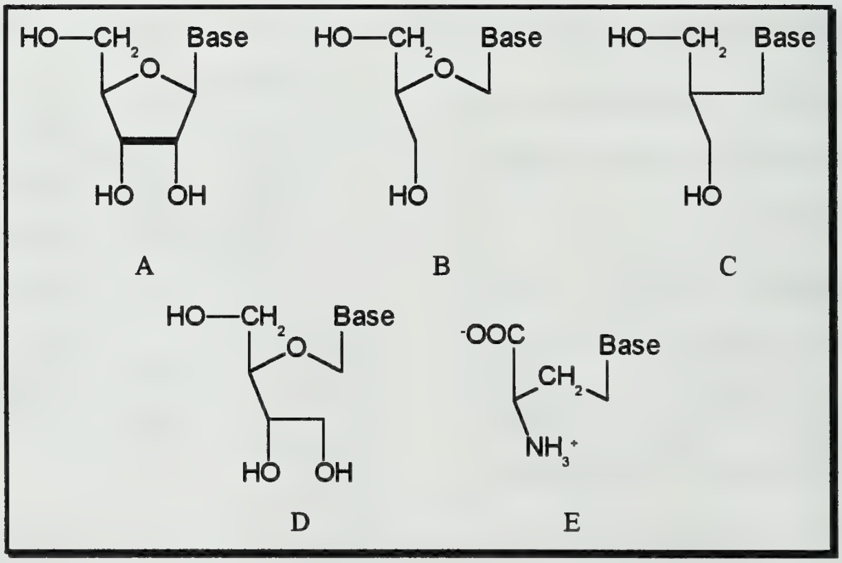


Figure 7: Alternative backbones, A = nucleoside, B = glycerol derived acyclonucleoside, C = acrolein-derived nucleoside, D = erythritol-derived acyclnucleoside, E = aspartate-derived nucleoside analogue

CONCLUSION

Certainly, compounds such as HCN, H_2CO, purines, and numerous amino acids were available on early earth. However, whether these combined to directly yield a fully self-assembled, self-replicating, information storage and transfer system remains to be proven. The primordial information storage and transfer system may never be found and in principal could never be proven. Many of the current chemical theories attempting to identify the path to such a system lack experimental support and are certainly unable to arrive at biotic life. The recent discoveries of ribozymes have placed RNA prior to proteins and DNA on an evolutionary time-scale, but the leap from the "primordial soup" to RNA is difficult to envision. The PNA templates have shown that it is possible for alternative backbones in "RNA-like" molecules to function in a similar manner to RNA, but much more data is needed before any one theory becomes a standard model for prebiotic chemical evolution.

References

1) Levine, J. S.; Augustsson, R. R. *Origins of Life* **1985,***15*, 299-318.
2) Oparin, A. I. *Adv. Enzymol.* **1965,** *27*, 347-380.
3) Dyson, R. J. *J. Molec. Evol.* **1982,** *18*, 344-350.
4) Kauffman, S. A. *J. Theor. Biol.* **1986,** *119*, 1-24.
5) Ferris, J. P.; Hagan, W. J. *Tetrahedron* **1984,** *40*, 1093-1120.
6) Ferris, J. P.; Hagan, W. J., *Tetrahedron* **1984,** *40*, 1093-1120.
7) Robertson, M. P.; Miller, S. L., *Science* **1995,** *268*, 702-705.
8) Ferris, J. P.; Orgel, L.E., *J. Amer. Chem. Soc.* **1966,** *88*, 1074. Sanchez, R. A., Ferris, J. P.; Orgel, L. E. *J. molec. Biol.* **1967,** *30*, 223-253.
9) Sanchez, R. A., Ferris, J. P.; Orgel, L.E. *Science* **1966,** *154*, 784-785.
10) Ferris, J. P.; Joshi, P. C. *J. Org. Chem.* **1979,** *44*, 2133-2137.
11) Robertson, M. P.; Miller, S. C. *Nature* **1995,** *375*, 772-774.
12) Shapiro, Robert *Origins of Life and Evolution of the Biospher,* **1995,** *25*, 83-98.
13) Mizuno, T.; Weiss, A. H. *Adv. Carbohyd. Chem. Biochem.* **1974,** *29*, 173-227.
14) Wiener, A. M.; Maizels, N. *Proc. Nat. Acad. Sci.* **1987,** *84*, 7383-7387.
15) Zielinski, W. S.; Orgel, L. E. *Nature* **1987,** *327*, 346-347.
16) Nielsen, P. E., Egholm, M., Berg, R. H., Buchardt, Ole *Science* **1991,** 1497-1500. 17) Nielsen, P. E., Egholm, M., Berg, R. H., Buchardt, Ole *Nature* **1993,** *365*, 566-568. Nielsen, P.E., Egholm, M., Berg, R. H., Buchardt, Ole *Proc. Nat. Acad. Sci.* **1993,** *90*, 1667-1670.

Supplementary References

1. Wetherill, G. W. *Science* **1985,** *228*, 877-889.
2. Schopf, J. W. and Packer, B. M. *Science* **1987,** *237*, 70-73.
3. Schidlowski, M. *Nature* **1988,** *333*, 313-318.
4. Maher, K. A.; Stevenson, D. J. *Nature* **1988,** *331*, 612-614.
5. Sleep, N. H., Zahnke, K .J., Kasting, J. F.; Morowitz, H. J. *Nature* **1989,** *342*, 139-142.

6. Cairns-Smith, A. G. *J. Theor. Biol.* **1966,** *10*, 53-88.
7. Cairns-Smith, A. G. *Genetic Takeover and the Mineral Origins of Life*. **1982** Cambridge University Press.
8. Wachtershauser, G. *System. Appl. Microbiol.* **1988,** *10*, 207-210.
9. Wachtershauser, G. *Micribiol. Rev.* **1988,** *52*, 452-484.
10. Wachtershauser, G. *PNAS,* **1990,** *87*, 200-204.
11. Wachtershauser, G. *Prog. Biophys. Mol. Biol.* **1992,** *58*, 85-201.
12. de Duve, Christian *Blueprint for a Cell.*
13. Woese, C. R. The Genetic Code: *The Molecluar Basis for Genetic Expression*. **1967,** New York: Harper and Row.
14. Orgel, L. E. *J. Mol. Biol.* **1968,** *38*, 381.
15. Kruger, K. Grabowski, P. J. Zaug, A. J., Sands, J., Gottschling, D. E.; Cech, T.R. *Cell* **1982,** *31*, 147.
16. Inoue, T.; Orgel, L. E. *Science* **1993,** *219*, 859-62.
17. Kruger, K. Orgel, L. E. *Cell* **1982,** *31*, 147-157.
18. Peebles, C. L., Mecklenberg, K. L., Perlman, P. S., Tabor, J.; Cheng, H.L. *Cell* **1986,** *44*, 213-223.
19. Uhlenbeck, O.C. *Nature,* **1987,** *328*, 596-600. Zaug, A.J. and Cech, T.R. *Science* **1986,** *231*, 470-475.
20. Bartel, D.P., Szostak, J.W. *Science* **1993,** *261*, 1411-1418.

UNIVERSITY OF ILLINOIS-URBANA
Q.547IL6S C001
ORGANIC SEMINAR ABSTRACTS URBANA
1995-96:2
3 0112 025513968

.547
ℓ 6s
996-97:1

ORGANIC SEMINAR ABSTRACTS
1996-97, SEMESTER I

University of Illinois

Department of Chemistry
Box 68 Roger Adams Laboratory
600 South Matthews Avenue
Urbana, Illinois 61801

January, 1997

Copyright © by the Board of Trustees of the University of Illinois

NOTICE: Return or renew all Library Materials! The *Minimum* Fee for each Lost Book is $50.00.

The person charging this material is responsible for its return to the library from which it was withdrawn on or before the **Latest Date** stamped below.

Theft, mutilation, and underlining of books are reasons for disciplinary action and may result in dismissal from the University.
To renew call Telephone Center, 333-8400

UNIVERSITY OF ILLINOIS LIBRARY AT URBANA-CHAMPAIGN

CHEMISTRY LIBRARY

UNIV
ILLIN
AT URBA
CH

L161—O-1096

Q.547

SEMINAR TOPICS

Semester I, 1996-97

Page

The Molecular Basis of Human Immunodeficiency Virus Type-1 (HIV-1) Protease Drug Resistance 1
Arvin Gee

The Syntheses of Balanol: A Potent Inhibitor of Protein Kinase C 9
Stephen P. Gitto

Catalytic Ring-Closing Metathesis 17
D. Scott Thompson

Synthesis of (±) Gelsemine 25
Brian Garcia

Palladium Catalyzed Aromatic Aminations: A New Methodology for the Conversion of Aryl Bromides and Iodides to Aryl Amines 33
Michael D. Curtis

Recent Advances in Studies on the Potential Role of Low Barrier Hydrogen Bonds in Enzymatic Catalysis 41
Pek Y. Chong

Carbon-Carbon Bond Formations Mediated by Samarium Diiodide 49
Ryan B. Prince

Tetraethynylethene: A Molecular Architect's Approach to Carbon Rich Frameworks 57
Gregory N. Tew

Mechanism of Asymmetric Induction for Carbenoid Transformations Using Chiral Rhodium(II) Catalysts....65
David R. Anderson

Enantioselective Transformations Catalyzed by Novel Heterobimetallic Complexes: Lanthanum-M-1,1'-Bi-2-Naphthol (M = Li, Na, K)....73
Xiao Yu

Molecular Imprinting - A Practical Tool or Novel Curiosity?....81
Ned Zimmerman

THE MOLECULAR BASIS OF HUMAN IMMUNODEFICIENCY VIRUS TYPE-1 (HIV-1) PROTEASE DRUG RESISTANCE

Reported by Arvin Gee September 9, 1996

INTRODUCTION

Human immunodeficiency virus type 1 (HIV-1) is the primary infectious agent leading to acquired immunodeficiency syndrome (AIDS). HIV-1 infection and the resulting AIDS is a life threatening disease and the first epidemic to occur on a worldwide basis. The World Health Organization estimates that 14 million people worldwide are currently infected with HIV-1 and that this number will increase to 40 million people by the year 2000.[1] HIV-1 is transmitted through sexual contact, body fluid contact, or by placental transfer from a mother to her child.[2]

In an effort to find a cure for AIDS, HIV research has focused on the molecular biology of HIV-1 and on antiviral therapies. Investigations of antiviral compounds have concentrated primarily on three essential HIV-1 enzymes, reverse transcriptase, integrase and protease. All three are essential enzymes in the HIV-1 life cycle and in fact, the first clinically useful antiviral drugs were the dideoxynucleoside reverse transcriptase inhibitors-- 3'-azido-2',3'-dideoxythymidine (AZT), 2',3'-dideoxycytidine (ddC), and 2',3'-dideoxyinosine (ddI).[2] Although initially successful, these agents have encountered two major shortcomings: patient toxicity to the dideoxynucleosides and the development of drug resistant viral mutants.

Drug strategies directed against HIV-1 protease have centered on the inhibition of the enzyme, but these therapies have also been hampered by the emergence of drug resistant mutant enzymes. This abstract will describe some of the protein mutations and the resulting changes in protein structure which afford HIV-1 protease drug resistance. This information can provide a basis of understanding for the development of rational strategies for overcoming this resistance.

BACKGROUND

Molecular Biology and Life Cycle of HIV-1

HIV-1 is a member of the retrovirus family, which includes HIV-2 and simian immunodeficiency virus (SIV). Viruses in this family are distinguished by having an RNA genome and two molecules of reverse transcriptase.[3] The single stranded RNA genome encodes for 9 genes which in turn code for 15 proteins. Three of these proteins, reverse transcriptase, integrase, and protease, all of

Copyright © 1996 by Arvin Gee

which are encoded by the gag-pol gene, are current targets for drug therapy. In the mature virion, the diploid RNA genome is located inside a nucleocapsid along with several of the HIV-1 essential proteins. This nucleocapsid is in turn surrounded by a lipid bilayer which embeds the two HIV-1 surface proteins, gp120 and gp41, along with host surface proteins. Figure 1 shows a diagrammatic sketch of the HIV-1 structure. Electron microscopy has shown mature virus particles to be icosahedral, with 72 spikes formed by the HIV-1 surface proteins.[2]

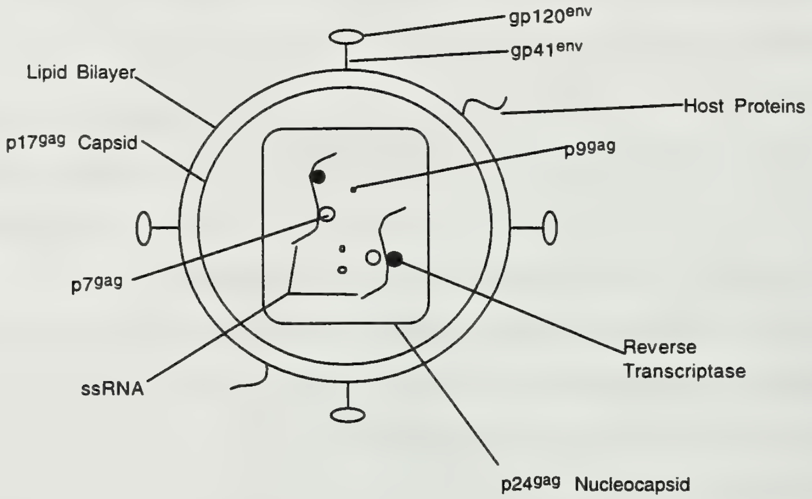


FIGURE 1. HIV-1 Structure.[1,2] ssRNA = single stranded ribonucleic acid

HIV-1 primarily infects CD4$^+$ T lymphocytes and monocytes.[2] These immune system cells use the CD4 receptor to interact with a surface protein, the class II major histocompatibility complex (MHC-II). MHC-II is used by cells to present foreign peptidic antigens to the CD4$^+$ cell to generate an immune response. The HIV-1 surface proteins, gp120 and gp41, mimic MHC-II and specifically interact with the CD4 receptor. This does not generate an immune response but rather allows the virus to gain entry into the cell. Once in the cell, the genomic viral RNA is quickly exposed and converted to double-stranded DNA by reverse transcriptase. The integrase protein then splices the viral DNA into the host cell's DNA. This provirus then enters its latent phase, which can last for several years until the provirus is reactivated by an unknown factor. At this point, the infected cell begins to make viral proteins and the viral protease comes into play. The gag and gag-pol genes are translated into large immature polyproteins which must be cleaved into mature proteins by HIV-1 protease in order to function. Failure of the protease to cleave the polyproteins results in non-infectious viral particles. Protease inhibitors are designed to prevent the polyprotein cleavage and thus result in the production of only non-infectious viral particles thereby arresting the disease progression. However, the efficacy of an inhibitor may be compromised by mutation of the protease.

Mutations in the HIV-1 genome

HIV-1 reverse transcriptase, responsible for transcription of the viral single stranded RNA into viral double stranded DNA, has very low fidelity since it has no proofreading function. It has been calculated that, in a patient infected by HIV-1, as many as 10^9 new cells are infected daily.[4] Based upon the low fidelity of transcription and the high celluar infection rate, Ridky and Leis estimate that every possible point mutation in the proteins occurs between 10^4 and 10^5 times per day per infected individual.[5] The majority of point mutations lead to non-infectious viral particles, but some of the mutations do lead to infectious viral particles that continue to propagate and carry along the accumulated mutations. The combination of a high rate of genomic mutation and pressure from the inhibitor can lead to the emergence of drug resistance.

HIV-1 PROTEASE

Structure and Mechanism

HIV-1 protease is an aspartate protease that is homologous to other proteases.[6] The active protein is a homodimer of two identical 99 amino acid subunits which are non-covalently associated. Each of the two subunits contributes a catalytic triad of Asp-Thr-Gly located at the base of the substrate binding cleft formed by the dimer interface and a highly mobile β-turn located over the active site, which serves as a flap that aids in holding the substrate in place. Figure 2 shows the β-turns and the active aspartate residues and their spatial relation to the substrate in the active site of HIV-1 protease.

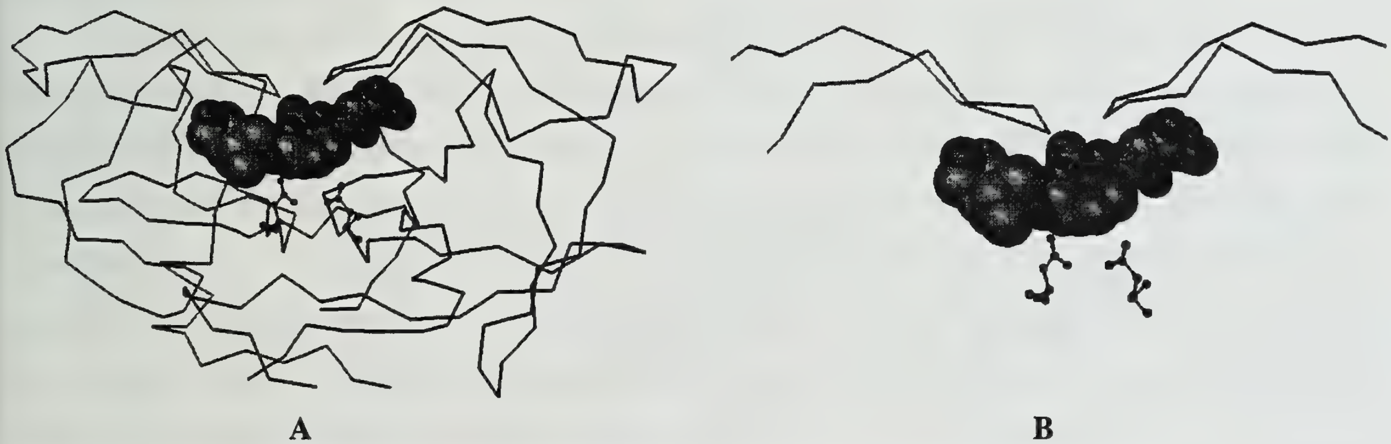


FIGURE 2. (A) A low-resolution face-on view of HIV-1 protease complexed with a substrate molecule, showing the catalytic aspartate residues. (B) The same view detailing the substrate, β-turns, and aspartate residues

Both the natural polyprotein substrate and many of the inhibitors, particularly the peptidomimetics, interact with the enzyme via a series of hydrogen bonds. Since there is substantial similarity in the hydrogen bonding patterns for both the substrate and inhibitors, the specificity of the enzyme is determined by the non-polar interactions between the binding subsites of the enzyme and the inhibitor sidechains which reside in them.[7] The observation that most of the protease mutations involve amino acids with aliphatic or aromatic side chains support this suggestion.

The protease dimer cuts the amide bond at specific sites on the gag and gag-pol polyproteins. The cleavage sites are summarized in Table 1. Information from molecular dynamics simulations and from isotopic substitution studies were used to elucidate the mechanism of the HIV-1 protease, which was postulated to act either by a general acid/general base or by a direct nucleophilic attack mechanism. Incorporation of ^{18}O into the hydrolysis products from $H_2{}^{18}O$ and kinetic rate changes due to ^{15}N, ^{2}H and ^{18}O support the general acid-general base mechanism depicted Figure 3.[8,9]

TABLE 1. Summary of HIV-1 Protease Cleavage Specificity[8,9]

N-Terminal Amino Acid Residue	C-Terminal Amino Acid Residue
Tyrosine	Proline
Leucine	Phenylalanine
Leucine	Alanine

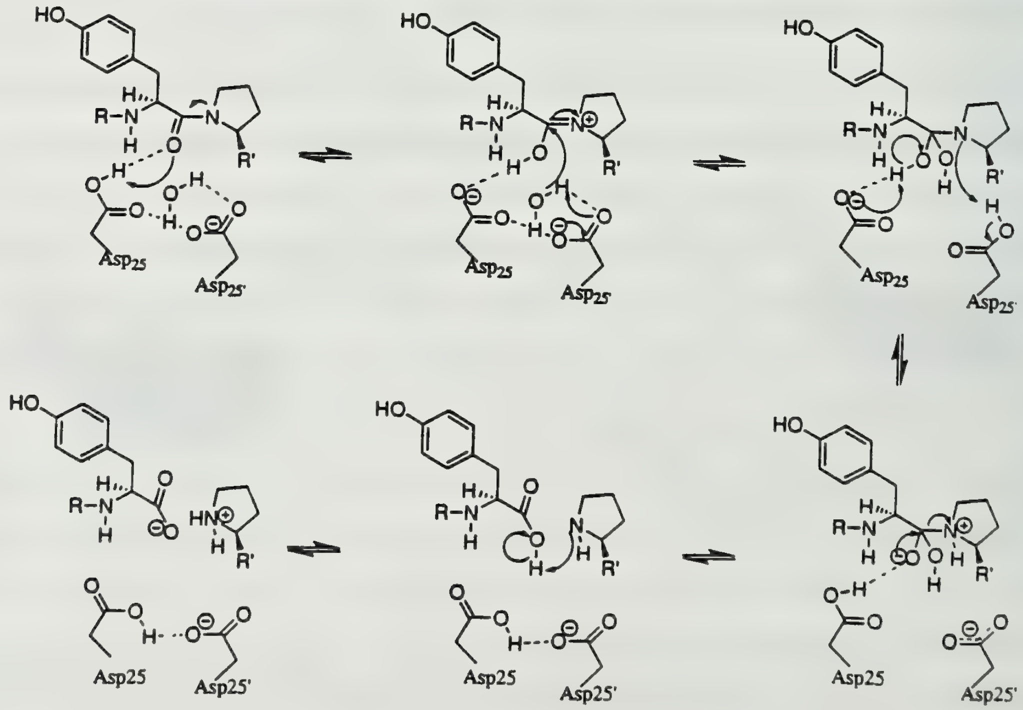
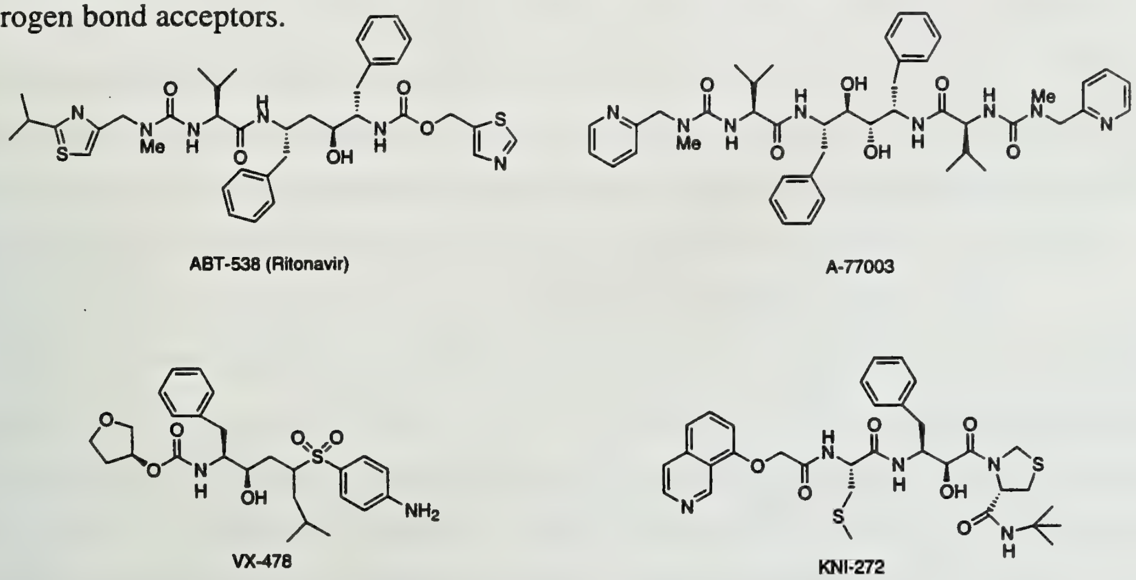


FIGURE 3. Proposed HIV-1 Protease hydrolysis mechanism.[8]

Inhibitors

Currently, there are several HIV-1 protease inhibitors in clinical trials as AIDS therapeutics. Figure 4 shows four such inhibitors. Many of the protease inhibitors are peptidomimetics and were designed using information about the structure and mechanism of HIV-1 protease. Inhibitors such as A-77003 exploit the C2 symmetry present in the protease molecule, and are considered to have equally optimized binding to both subunits of HIV-1 protease. Two features present in all the clinically useful inhibitors are the presence of large non-polar side chains and the presence of both hydrogen bond donors and hydrogen bond acceptors.



ABT-538 (Ritonavir)

A-77003

VX-478

KNI-272

FIGURE 4. Some clinically useful HIV-1 Protease inhibitors.[7]

HIV-1 Protease Mutations

It has been found, in cell culture and in untreated HIV-infected humans, that inhibitor-resistant HIV-1 proteases exist at a low level.[10, 11] This basal level of resistant mutants is produced because HIV-1 reverse transcriptase introduces an estimated 5-10 errors into the entire HIV-1 genome during each transcription event.[3] The errors created during transcription involve a single nucleotide substitution which may lead to a new codon in the reading frame that translates into an amino acid substitution in the protein. It has been calculated that if a mutant, in the absence of inhibitor, exists at a level that is 0.5% of the wild-type virus, it can become the dominant phenotype after 1000 replications in the presence of an inhibitor which suppresses the wild-type virus.

Different inhibitors select for a different series of mutations because of the variations in the specific interactions between each inhibitor and the enzyme. As anticipated, point mutations in the protease enzyme emerge in an ordered and sequential fashion.[12] The sequence of point mutation emergence was studied by following patients in a clinical study of ritonavir (ABT-538). Molla, et. al., found that a mutation of valine-82 to alanine, threonine or phenylalanine occurred first, followed by isoleucine-54 to valine and to a lesser extent, threonine. This mutation is then followed by alanine-71 to valine and to a lesser extent, threonine.[12] Mutations at nine other positions were noted, but these did not occur enough to be considered statistically valid. It can be surmised from these results that valine-82, isoleucine-54 and alanine-71 are contact or near contact residues between ritonavir and the protease enzyme. Most of the resistance mutations that have been observed occur in one of the 3 unique binding subsites.[7] Each subunit of the HIV-1 protease contains all three of these unique binding subsites. Figure 5 shows the inhibitor A-77003 in relation to the binding subsites of HIV-1 protease along with the C2 axis of symmetry. A similar study with other HIV-1 protease inhibitors demonstrated a unique pattern of mutations for each inhibitor, as is summarized in Table 2. These patterns of mutations allow one to map out the contact residues in the binding subsites between each inhibitor and the enzyme.

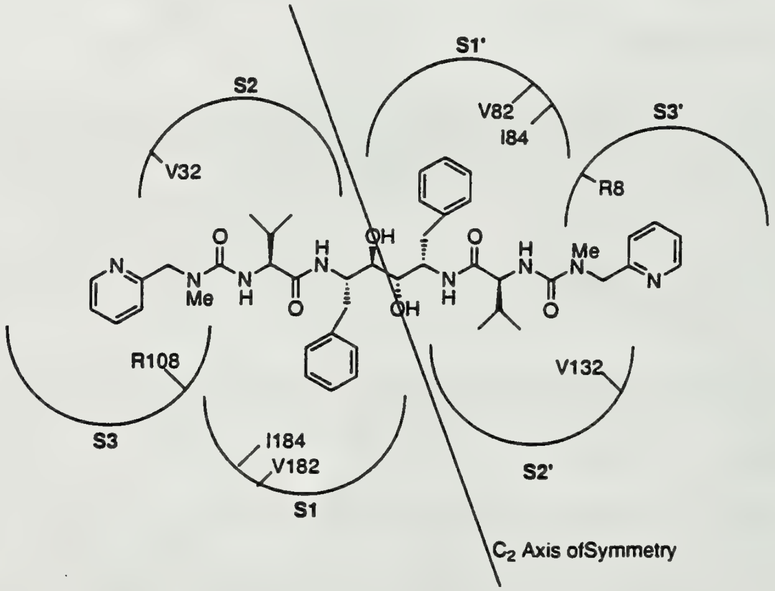


FIGURE 5. Schematic diagram of A-77003 interacting with the binding subsites of HIV-1 Protease, showing the amino acids known to mutate[7]

TABLE 2. Mutation pattern induced by various inhibitors[7,13,14]

	HIV-1 Protease Inhibitor			
Mutation	A-77003	KNI-272	ABT-538	Ro31-8959
R8Q	+			
R8K	+			
V32I	+	+		
M46I			+	
I47V				
G48V	+			+
V82A	+			
V82I	+			
V82F			+	
I84V		+	+	+

Curiously, not all of the resistance providing mutations occur in the binding site of the enzyme. The mutation of methionine-46 to isoleucine decreases the mobility of the hinge for the flap and is thus believed to hold the enzyme substrate in the active site more tightly that in the wild type.[13] These non-binding site mutations are likely the products of secondary mutation events rather than primary mutation events, as they do not provide any resistance advantage as compared to the wild type enzyme.

The mutant enzymes, though resistant towards the various inhibitors, do not necessarily carry the same kinetic profile as the wild-type enzyme. Many of the mutations that yield resistance also create a drop in the enzymatic efficiency of the enzyme. Some mutate enough to become inactive. Multiple mutations can and do occur, and these can sometimes restore enzyme activity to near wild-type levels while maintaining a reduced sensitivity towards a particular inhibitor by 30- to 100-fold.[14] The secondary mutations may act by holding the polyprotein substrate in the binding site more tightly than does a single mutation in the protease. Methionine-46 mutation is believed to be one such case. Multiple mutations can also provide the protease with cross-resistance towards multiple inhibitors.[5,15,16,17]

When a mutation occurs in the protease, the mutation occurs symmetrically to both subunits of the homodimeric HIV-1 protease. This has important implications for the continued susceptibility of HIV-1 protease towards inhibitors that are designed to exploit the C2 symmetry of the protease. These inhibitors, such as A77003, were designed to be C2 symmetrical, so that their binding would be enhanced. However, when the active site mutates, the affinity of the protease for both halves of the inhibitor is decreased by the same amount. If the inhibitor were not C2 symmetric, then the protease may lose its affinity for half of the inhibitor, yet retain its affinity for the other half of the inhibitor.

As more structural detail emerges about the interaction of resistant mutants with various inhibitors, one can begin to target new inhibitors to the resistant mutants. Cross-resistant mutants can also be beneficial if one can design new inhibitors to be less optimized towards a particular mutant and rather be targeted for multiple mutants. The C2 symmetric inhibitors, such as A-77003, experiences a decrease in binding affinity to both subunits when a resistance-conferring mutation occurs. But if one were to make a “hybrid” inhibitor, with one half optimized for the wild-type protease and the other half optimized for a known mutant, it may be the case that the inhibitor is active towards both the wild type and the mutant.

CONCLUSION

Decreased sensitivity of the HIV-1 protease towards inhibitors has its basis in the amino acid substitution from mutations. The pattern of these mutations varies with each inhibitor, but in each case they change the molecular interactions between the inhibitor and the protease enzyme. Since the specific mutations are selected by the inhibitor present to apply pressure, the use of multiple inhibitors may prevent the emergence of drug resistant mutants. Also by understanding the specific molecular interactions between an inhibitor and the protease mutants, future generations of inhibitors can be designed around the finite number of viable mutants. The interactions between future inhibitors and the resistant enzymes can be engineered to avoid interacting with those amino acid residues that are known to have a high frequency of mutations.

REFERENCES

1. Pantaleo, G.; Graziosi C; Fauci A. S.; *N. Engl. J. Med* **1993**, 328, 327-335.
2. Greene, W. C. *N. Engl. J. Med.* **1991**, 324, 308-317.
3. Kuby, J. *Immunology, 2nd ed.*, W. H. Freeman: New York, 1994; p 526.
4. Coffin, J.M., *Science* **1995**, 267, 483-489.
5. Ridky, T.; Leis, J. *J Biol. Chem.* **1995**, 270, 29621-29623.
6. Sardana, V. V.; Schlabach, A. J.; Graham, P.; Bush, B. L.; Condra, J. H.; Culberson, C.; Gotlib, L.; Graham, D. J.; Kohl, N. E.; LaFemina, R. L.; Schneider, C. L.; Wolanski, B. S.; Wolfgang, J. A.; Emini, E. A. *Biochemistry* **1994**, 33, 2004-2010.
7. Erickson, J. W.; Burt, S. K. *Annu. Rev. Pharmacol. Toxicol.* **1996**, 36, 545-571.
8. Chatfield, D. C.; Brooks, B. R. *J. Am. Chem. Soc.* **1995**, 117, 5561-5572.
9. Meek, T. D.; Rodriguez, E. J.; Angeles, T. S. In *Methods in Enzymology*; Kuo, L. C., Shafer J. A., Eds.; Academic: San Diego, 1994; Vol. 241; pp 127-156.
10. Rose, R. E.; Gong, Y-F.; Greytok, J. A.; Bechtold, C. M.; Terry, B. J.; Robinson, B. S.; Alam, M.; Colonno, R. J.; Lin, P-F. *Proc. Natl. Acad.* **1996**, 4, 1648-1653.
11. Lech, W. J.; Wang, G.; Yang, Y. L.; Chee, Y.; Dorman, K.; McCrae, D.; Lazzeroni, L. C.; Erickson, J. W.; Sinsheimer, J. S.; Kaplan, A. H. *J. Virology*, **1996**, 3, 2038-2043
12. Molla, A.; Korneyeva, M.; Gao, Q.; Vasavanonda, S.; Schipper, P. J.; Mo, H-M.; Markowitz. M.; Chernyavskiy, T.; Niu, P.; Lyons, N.; Hsu, A.; Granneman, G. R.; Ho, D. D.; Boucher, C. A. B.; Leonard, J. M.; Norbeck, D. W.; Kempf, D. J. *Nature Medicine*, **1996**, 2, 760-766.
13. Markowitz, M.; Mo, H.; Kempf, D. J.; Norbeck, D. W.; Bhat, T. N.; Erickson, J. W.; Ho, D. D. *J. Virology*, **1995**, 2, 701-706.
14. Jacobsen, H.; Yasargil, K.; Winslow, D. L.; Craig, J. C.; Kröhn, A.; Duncan, I. B.; Mous, J. *Virology*, **1995**, 1, 527-34.
15. Chen, Z.; Li, Y.; Schock, H. B.; Hall, D.; Chen, E.; Kuo, L. C. *J. Biol. Chem.*, **1995**, 37, 21433-21436.
16. Tisdale, M.; Myers, R. E.; Maschera, B.; Parry, N. R.; Oliver, N. M.; Blair, E. D. *Antimicrob. Agents and Chemother.*, **1995**, 8, 1704-1710.
17. Condra, J. H.; Schleif, W. A.; Blahy, O. M.; Gabryleski, L. J.; Graham, D. J.; Quintero, J. C.; Rhodes, A.; Robbins, H. L.; Roth, E.; Shivaprakash, M.; Titus, D.; Yang, T.; Teppler, H.; Squires, K. E.; Deutsch, P. J.; Emini, E. A. *Nature*, **1995**, 6522, 569-571.

THE SYNTHESES OF BALANOL: A POTENT INHIBITOR OF PROTEIN KINASE C

Reported by Stephen P. Gitto

September 12, 1996

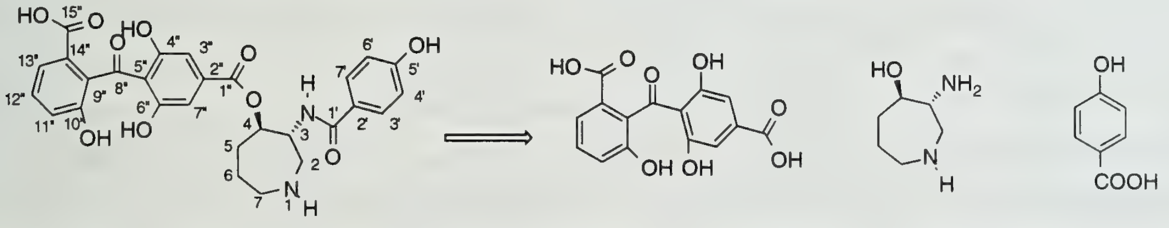
INTRODUCTION

Balanol **1**, a metabolite recently isolated from three different fungi,[1,2] has remarkable inhibitory effects on protein kinase C (PKC) at concentrations as low as 3-4 nanomolar *in vitro*.[1] PKC has received much attention because of its role in the mechanism of cell surface signal transduction as a serine/threonine kinase in the phosphoinositide cascade. The kinase has been linked to critical cellular responses such as cell proliferation and gene transcription, and been implicated in the cause of many potentially fatal disorders including uncontrolled cell proliferation producing tumors.[3]

The total synthesis of balanol has given access to a product of otherwise low natural abundance. The total synthesis has also provided the framework for synthetic studies of analogs which have been used to establish structure-activity relationships and search for more powerful inhibitors.

SYNTHESIS

Retrosynthetic analysis of balanol **1** shows obvious disconnections of the amide and the ester to provide a highly substituted benzophenone **2**, 3-amino-4-hydroxy-hexahydroazepine **3**, and 4-hydroxybenzoic acid **4**. The benzophenone **2** has a very hindered carbonyl in the presence of two heavily functionalized aryl rings. The azepine **3**, a seven-membered heterocycle, contains two contiguous stereocenters. This analysis shows the syntheses of balanol can be simplified to independent syntheses of its subunits.



1 2 3 4

Benzophenone Unit

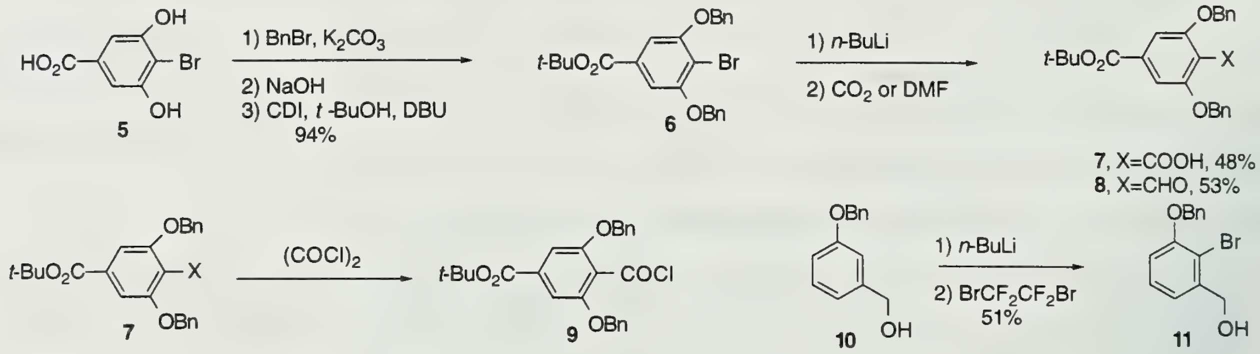
There have been three syntheses of the benzophenone fragment.[4-6] The main problems associated with the benzophenone syntheses are the coupling and the protection. For such a highly functionalized compound it was desirable to seek protection that could be added and removed in one step. The

Copyright © 1996 by Stephen P. Gitto

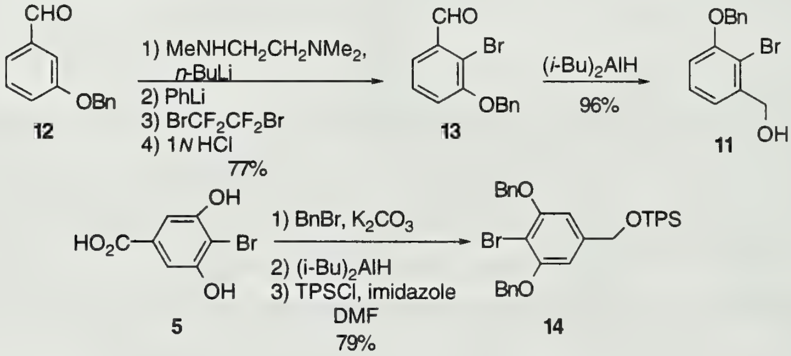
benzyl group served this purpose well.

The syntheses of key intermediates **9** and **14** were carried out from commercially available **5** by Lampe and Hughes[4] (Scheme I) and Nicolau[6] (Scheme II). Likewise, **11** was prepared from the readily available **10** by organolithium methodology.

Scheme I



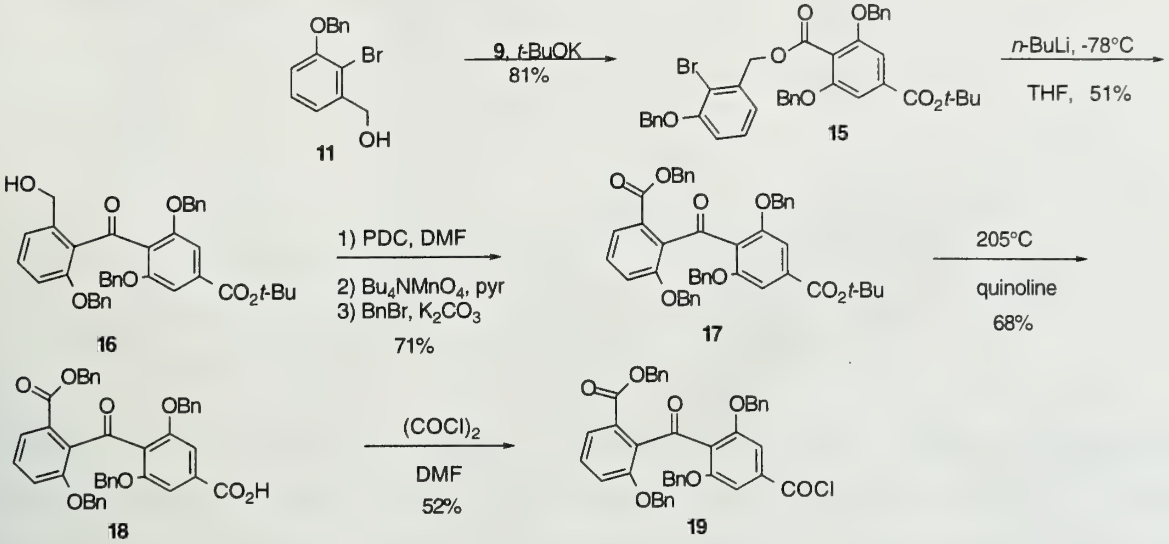
Scheme II



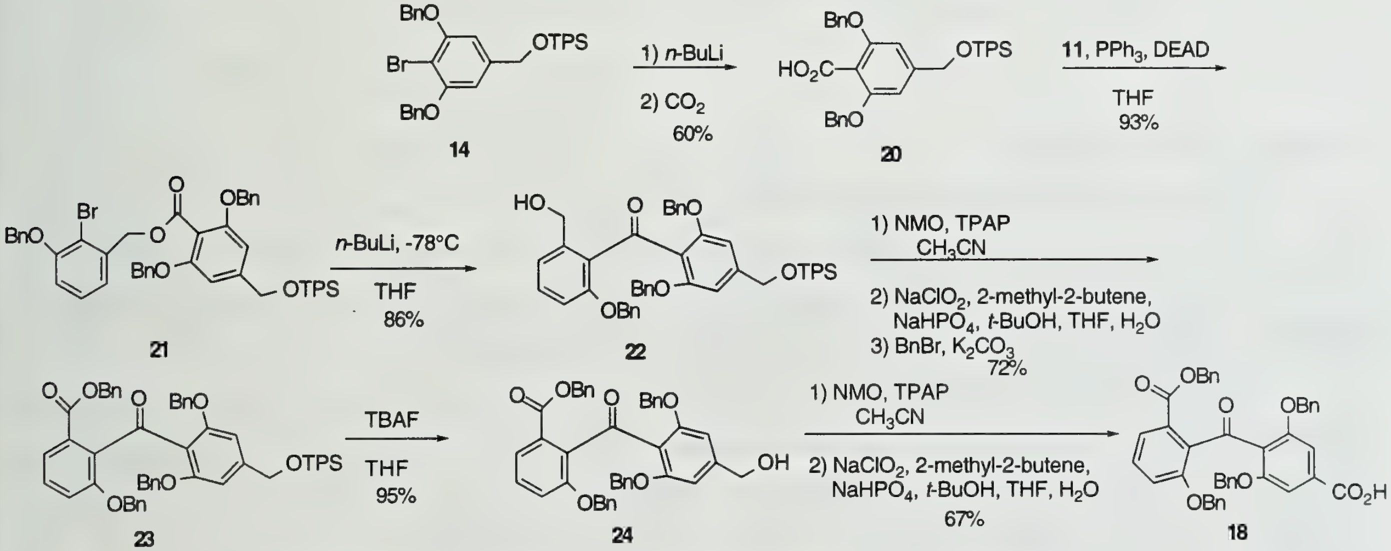
Addition of the lithiated or stannylated derivatives of **11** to acid chloride **9** did not yield any benzophenone. Addition of a lithiated derivative of **11** to the aldehyde **8** went well, but oxidation of the alcohol to the ketone **17** was quite problematic due to both steric hindrance and the instability of the compound under oxidation conditions. The curious observation of the aldehyde being reactive and the acid chloride being unreactive was attributed to the bulk of the halide twisting the carbonyl out of the plane of the aromatic ring, while the aldehyde remained planar.[4b] In order to confirm this hypothesis, a computer simulation was done in Macromodel using MM2 force field. The minimizations showed the aldehyde to be out of plane by about 10° and the acid chloride twisted out of plane by approximately 40°. The benzyloxy substituents in **9** effectively block the Bürgi-Dunitz trajectory for the acid chloride,[7] and increase steric crowding in the transition state leading to the tetrahedral intermediate.

In order to solve this steric problem, Lampe and Hughes[4] (Scheme III) and Nicolau[6] (Scheme IV) used an intramolecular nucleophilic rearrangement. The esters **15** and **21** were prepared, and lithiation initiated an anionic Fries rearrangement which yielded the desired benzophenones in 51% yield for **16** and 86% yield for **22**. This rearrangement is analogous to the traditional Fries rearrangement, although this reaction is promoted by an anion instead of a Lewis acid and involves a benzyl alcohol instead of a phenol.[8] The reaction may not be convenient for large scale preparation because of the low temperature required to initiate lithium-halogen exchange without BuLi adding to the ester.

Scheme III

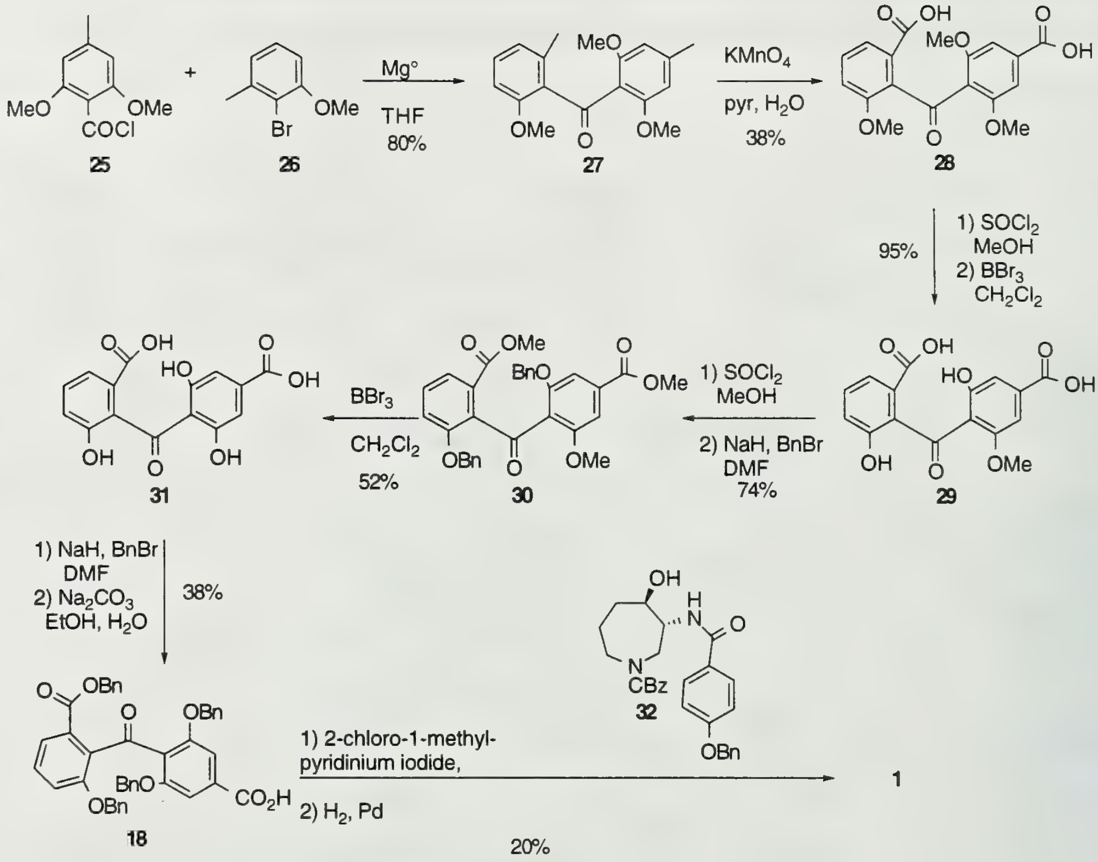


Scheme IV



Smaller protecting groups were employed to determine if intermolecular organometallic addition could be practical. Vicker[5] used methoxy groups (Scheme V). Addition of the Grignard derived from **26** to the acid chloride **25** and oxidation of the methyl groups as latent precursors of carboxylic acids gave **28**. Deprotection was impractical as incomplete removal resulted in insoluble **29**. Further reaction required reprotection with benzyl groups at a cost of three extra steps. Methoxymethyls were also used.[4c] Deprotection was not attempted, but probably would be impractical.

Scheme V

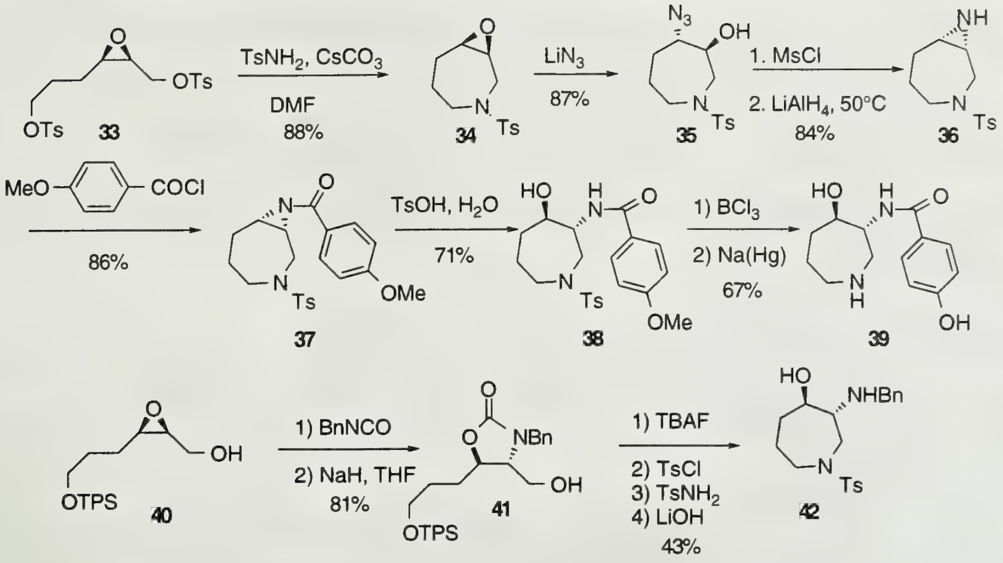


Azepine Unit

The azepine fragment was synthesized easily several times in racemic form.[4b,5,9-11] Studies showed that (-)-balanol **1** was at least 100 times more potent an inhibitor of PKC than (+)-balanol.[4b] The resolution of enantiomers was quite expensive and would at best yield only 50% active material. Enantioselective syntheses were necessary to improve yield and reduce cost.

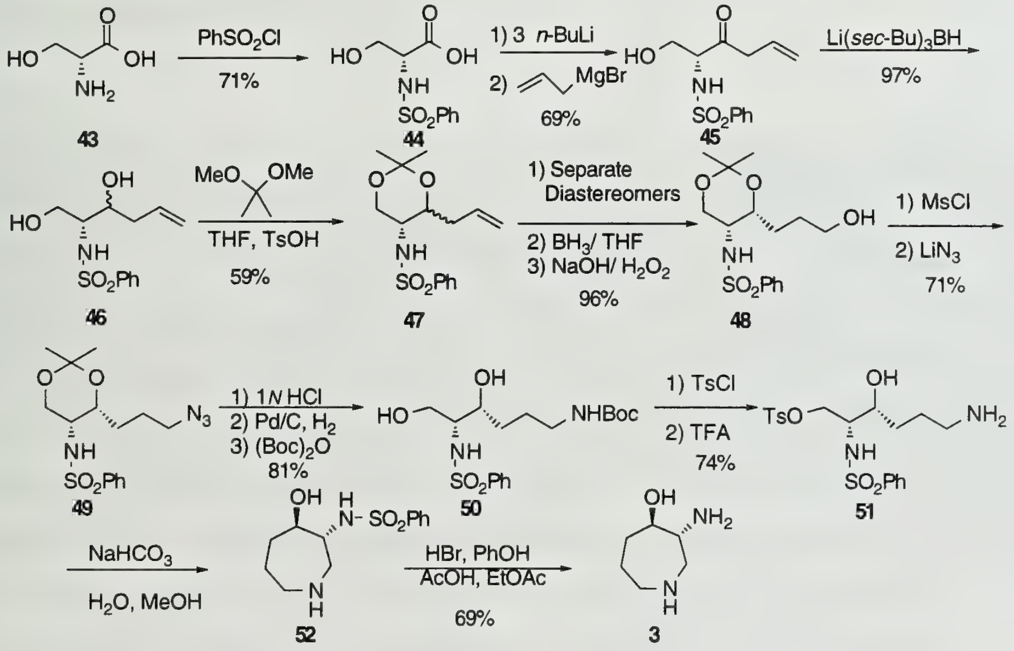
The main problems in the syntheses of the azepine are the formation of the seven-membered heterocycle and the establishment of the two contiguous stereocenters. The heterocycle was formed most effectively from acyclic precursors because ring expansions were often poor in yield.[11] Synthetic strategies differed in whether the stereochemistry was established before or after cyclization. One approach utilized a chiral epoxide **33** which was obtained by Sharpless asymmetric epoxidation. Tanner[12] cyclized **33** to **34** before opening the epoxide to azide **35.** However, **35** did not have the desired regiochemistry necessitating the functional group interconversion through aziridine **36** (Scheme VI). The problem was more properly addressed by intramolecular carbamate formation giving **41**.

Scheme VI



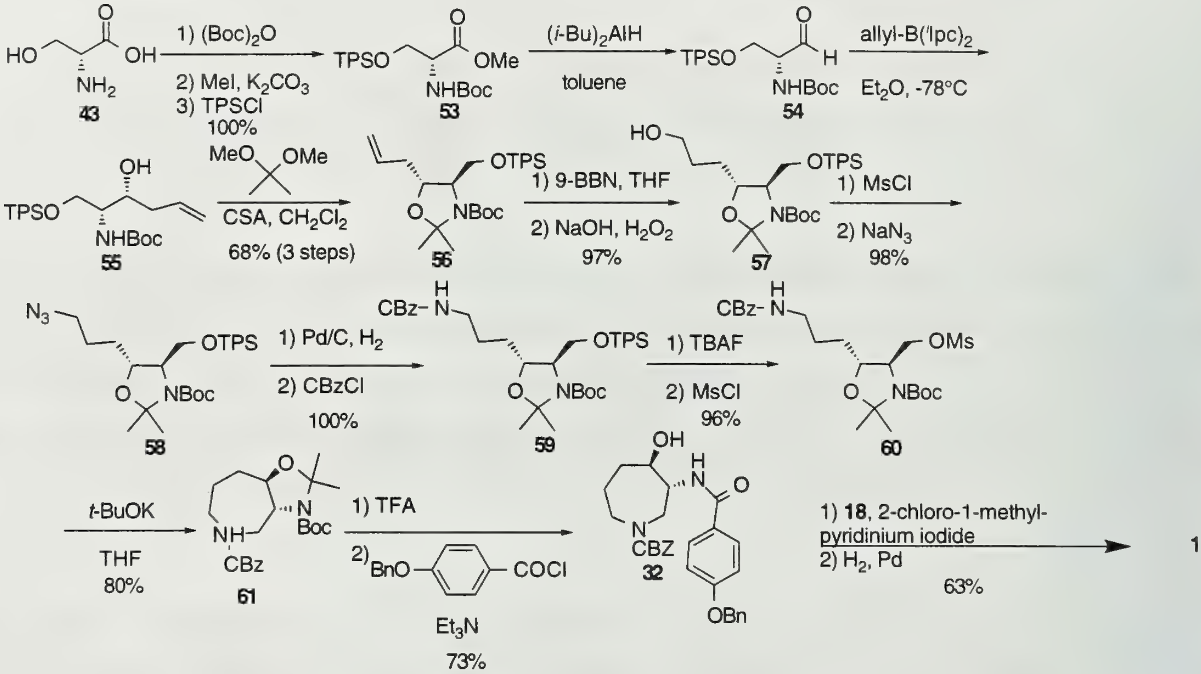
Three syntheses used 3*R*-hydroxylysine as a precursor.[4,6,11] König[11] (Scheme VII) and Nicolau[6] (Scheme VIII) both began with the commercially available D-serine. In each case the carbon skeleton was extended by means of allylation. König, who was synthesizing another natural product, used the method of Roemmele and Rapoport[13] in which the trianion of the N-protected serine was allylated and then reduced with an excellent selectivity of 25:1 of *R*,*R*:*R*,*S* for **46**.

Scheme VII



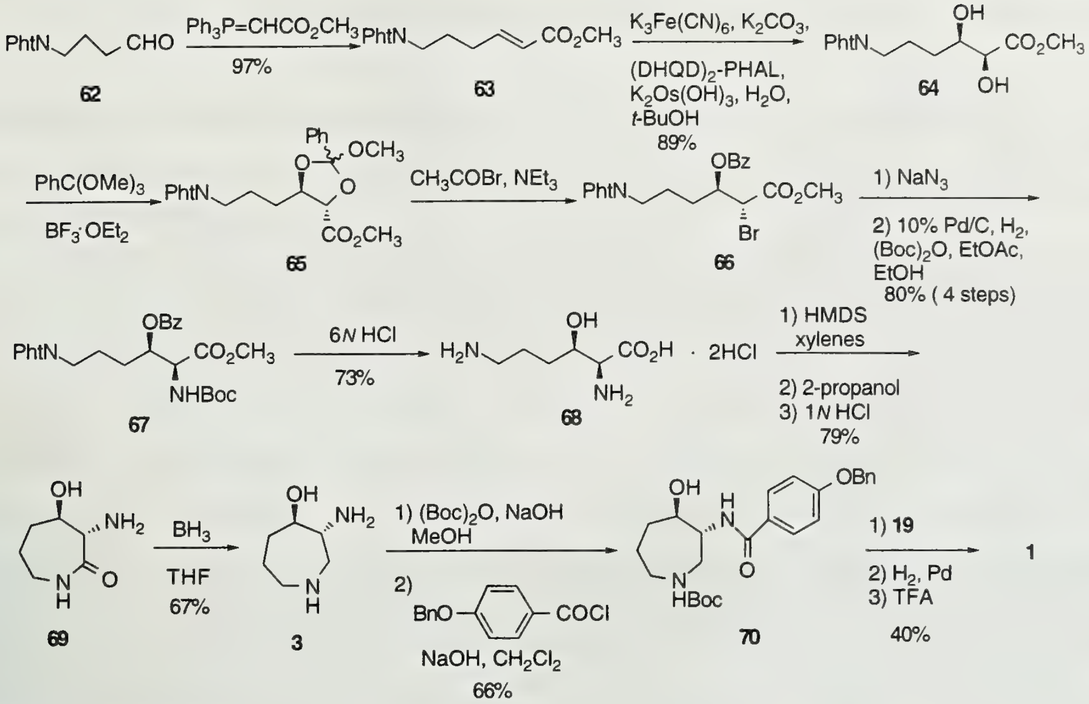
Such high selectivity is curious as similar attempts by both Nicolau[6] and Rapoport[13] were not as satisfactory. This method saves steps from usual protection, but is inconvenient for large scale preparation because of the necessity for low temperature. Nicolau used Brown's diisopinocampheylborane[14] as a chiral auxillary to effect the desired stereochemistry during allylboration of aldehyde **54** giving a diastereoselectivity of 12:1 for **55**. The selectivity was governed in a cyclic transition state by the chirality of the auxillary. Likewise, this allylation was done at low temperature which would be inconvenient to perform on large scale. Following allylation both syntheses required the separation of diastereomers by chromatography. An acetonide was used for protection prior to hydroboration and subsequent functional transformation led to protected amines **51** or **60** from which cyclization occurred both with and without the rigidity of the acetonide.

Scheme VIII



Lampe and Hughes began with achiral starting material, and used Sharpless asymmetric dihydroxylation followed by protection, formation of bromide **66** with acetyl bromide, and substitution by azide to yield **69** with the two stereocenters in place (Scheme IX).[4] This method allows scale-up except for the necessity of column chromatography. Cyclization to form the azepine **69** was done by means of an intramolecular acylation which resulted in complete epimerization at the 3-amino position.[4] With HMDS epimerization was limited to 7% after borane reduction and cleavage of silyl protection. In general the cyclization was best effected when a tosylate or mesylate was displaced through intramolecular alkylation of amine **51** or **60**.

Scheme IX



Combination of Subunits

The subunits were connected following their syntheses. The amide of the azepine **32** or **70** was formed with the preprotected 4-benzyloxy-bezoylchloride. The ester bond was formed by activating acid **18**. All of the protection was removed in one step in the case of **32**, but two steps were required in the case of **70** because of Boc protection.

Comparison

Lampe and Hughes synthesized balanol in 29 steps with 6.3% yield at an estimated cost of $52/gram. Nicolau used 32 steps with much more impressive yield of 22.9% at an estimated cost of $64/gram. Vicker, who did not perform an enantioselective synthesis of the azepine used 29 steps with 2.3% yield at an estimated cost of $668/gram. All three syntheses will have problems with adaption to very large scale. The benzophenone synthesis is most effectively done by Lampe and Hughes[4] and Nicolau.[6] Both routes have the necessity for benzyl protection. The steric consequences are dealt with by means of an intramolecular nucleophillic rearrangement. The azepine syntheses are varied. Enantioselective syntheses are necessary to improve yield and cost. Cyclic precursors typically required an additional separation of isomers because of achirality and usually involved a ring expansion. Acyclic precursors are clearly preferred because of the relative ease of establishing stereochemistry and forming the 7-membered ring. Beginning with chiral starting material leaves only one stereocenter to be fixed as in the sequences of Tanner, König, and Nicolau.[6,11,12] The most selective method is the reduction by König. The Lampe and Hughes method is most compatible with large scale, but both König and Nicolau use the most convenient starting material. The Tanner synthesis is effective because it does not require a separation of isomers after the epoxidation.

FUTURE WORK

Future work in this area will be centered on making balanol a better inhibitor. A structure-activity relationship based on numerous structural analogs has shown that the entire balanol skeleton is important.[15,16] None of the synthetic mimics have been able to perform *in vitro* as well as **1**.[16] The mechanism of action is a competitive inhibition of PKC for the binding of ATP.[2b,15] However, the viability of balanol as a drug is questionable. In cellular assays balanol fails to perform due to its inability to cross plasma membranes.[16] Current research focuses on creating analogs or prodrugs capable of showing good activity in cellular assays.

REFERENCES

1) Kulanthaivel, P.; Hallock, Y.; Boros, C.; Hamilton, S.; Janzen, W.; Ballas, L.; Loomis, C.; Jiang, J.; Katz, B.; Steiner, J.; Clardy, J. *J. Am. Chem. Soc.* **1993**, *113,* 6452.

2a) Kneifel, H.; König, W.; Loeffler, W.; Müller, R. *Arch. Microbiol.* **1977**, *113*, 121. b) Oshima, S.; Yanagisawa, M.; Katoch, A.; Fujii, T; Sano, T.; Matsukuma, S.; Furumai, T.; Fujiu, M.; Watanabe, K.; Yokose, K.; Arisawa, M.; Okuda, T. *J. Antibiotics* **1994**, *47*, 639.

3) Nishizuku, Y. *Nature (London)* **1984**, *308*, 693.

4a) Lampe, J.; Hughes, P.; Biggers, C.; Smith, S.; Hu, H. *J. Org. Chem.* **1994**, *59*, 5147. b) Lampe, J.; Hughes, P.; Biggers, C.; Smith, S.; Hong, H. *J. Org. Chem.* **1996**, *61*, 4572. c) Hollingshead, S.; Nichols, J.; Wilson, J. *J. Org. Chem.* **1994**, *59*, 6703. d) Hughes, P.; Smith, S.; Olson, J.; *J. Org. Chem.* **1994**, *59*, 5799.

5) Adams, C.; Fairway, S.; Hardy, C.; Hibbs, D.; Hursthouse, M.; Morley, A.; Sharp, B.; Vicker, N.; Warner, I. *J. Chem. Soc. Perkin Trans. 1* **1995**, 2355.

6a) Nicolau, K.; Bunnage, M.; Koide, K.; *J. Am. Chem. Soc.* **1994**, *116*, 8402. b) Nicolau, K.; Koide, K.; Bunnage, M. *Chem. Eur. J.* **1995**, *1*, 454.

7a) Bürgi, H.; Dunitz, J.; Shefter, E. *J. Am. Chem. Soc.* **1973**, *95*, 5065. b) Bürgi, H.; Dunitz, J.; Lehn, J.; Wipff, G. *Tetrahedron* **1974**, *30*, 1563.

8) Blatt, A.; *Org. React.* **1942**, *1*, 343.

9) Naito, T.; Torieda, M.; Tajiri, K.; Ninomiya, I.; Kighucgi, T. *Chem. Pharm. Bull.* **1996**, *44*, 624.

10) Hu, H.; Jagdmann, G.; Hughes, P.; Nichols, J. *Tet. Lett.* **1995**, *36*, 3659.

11) Müller, A.; Takyar, D.; Witt, S.; König, W. *Liebigs Ann. Chem.* **1993**, *6*, 651.

12) Tanner, D.; Almario, A.; Högberg, T. *Tetrahedron* **1995**, *51*, 6061.

13) Roemmele, R.; Rapoport, H. *J. Org. Chem.* **1989**, *54*, 1866.

14) Brown, H.; Bhat, K.; Randad, R. *J. Org. Chem.* **1989**, *54*, 1570.

15) Koide, K.; Bunnage, M.; Paloma, L.; Kanter, J.; Taylor, S.; Brunton, L.; Nicolau, K. *Chem. Biol.* **1995**, *2*, 601.

16a) Crane, H.; Menaldino, D.; Jagdmann, G.; Darges, J.; Buben, J. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2133. b) Heerding, J.; Lampe, J.; Darges, J.; Stamper, M. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1839. c) Hu, H.; Hollinshead, S.; Hall, S.; Kalter, K.; Ballas, L. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 973. d) Jagdmann, G.; Defauw, J.; Lai, Y.; Crane, H.; Hall, S.; Buben, J.; Hu, H.; Gosnell, P. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2015. e) Lai, Y.; Stamper, M. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2147. f) Lai, Y.; Menaldino, D.; Nichols, J.; Jagdmann, G.; Mylott, F.; Gillespie, J.; Hall, S. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2151. g) Lai, Y.; Mendoza, J.; Hubbard, F.; Kalter, K. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2155. h) Mendoza, J.; Jagdmann, G.; Gosnell, P. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2211.

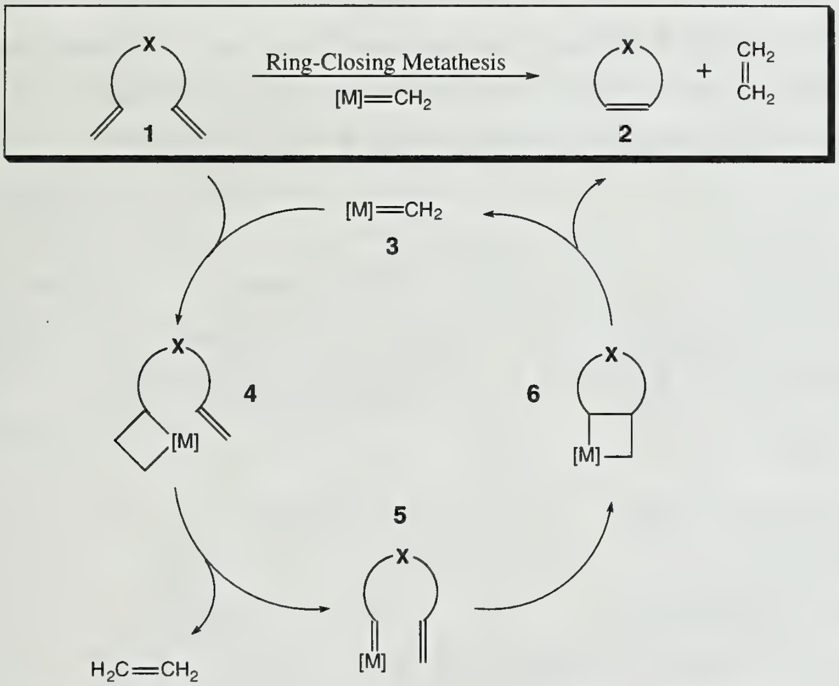
CATALYTIC RING-CLOSING METATHESIS

Reported by D. Scott Thompson September 23, 1996

INTRODUCTION

Catalytic olefin metathesis involves cleavage of a carbon-carbon double bond of an alkene, promoted by a metal alkylidene, with redistribution of the metal alkylidene fragments to form a new alkene.[1] Application of this process to provide cyclic alkenes has led to the development of Ring Closing Metathesis (RCM) as a catalytic method for ring closures. This reaction, illustrated by the transformation of **1** to **2** in Scheme I, offers substantial advantages over alternative methods. The key to this development has been the preparation of well defined, highly active, and functional group tolerant catalysts by the groups of Schrock and Grubbs. Since the initial findings, a number reports on the synthetic utility of this process have appeared in the literature.

Scheme I. Catalytic Cycle for Ring Closing Metathesis



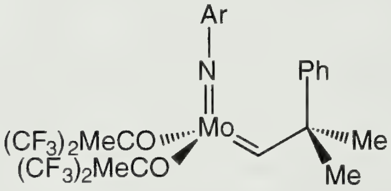
MECHANISM AND CATALYSTS

The mechanism of ring closing olefin metathesis is based on extensive studies of olefin metathesis and supported by evidence from work on ring opening metathesis polymerization.[2]

Copyright © 1996 by David Scott Thompson

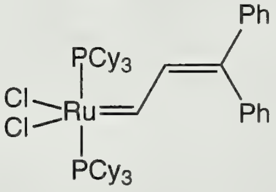
Scheme I illustrates the general catalytic cycle for RCM. The generally accepted mechanism involves initial [2+2] cycloaddition between the transition metal alkylidene **3** and one of the olefins of **1** to produce a metallacyclobutane intermediate **4**. Subsequent retrocyclization gives a new tethered metal alkylidene **5** with release of one equivalent of ethylene. The tethered metal alkylidene then undergoes intramolecular [2+2] cycloaddition to produce a new metallacyclobutane intermediate **6**. Retrocyclization regenerates free metal alkylidene, which continues to propagate, and releases the ring closed product, **2**.[3] As olefin metathesis is a reversible process, a delicate thermodynamic balance is needed in order for the reaction to proceed. Combined with the evaporative lose of ethylene, the fact that RCM generates two molecules from one should provide an entropic driving force for the reaction. The change in enthalpy, however, must be small for the reaction to proceed.

The three most commonly used catalysts for ring closing metathesis are **7**, **8**, and **9**. Catalyst **7**, synthesized by Schrock[4] and applied by Grubbs, was the first to show activity in ring closing metathesis for a variety of substrates.[5] However, this catalyst requires preparation under inert conditions and is unstable in air. The facile synthesis and increased oxidative stability of catalyst **8**, reported by Grubbs *et al.*[6], led to increased use of the RCM reaction. The stability of catalyst **8** has been demonstrated by its activity for ring opening metathesis polymerization in protic media and in RCM reactions in the presence of air.[7] Catalyst **9** is a recent entry which is more active and stable than **8**.[8]



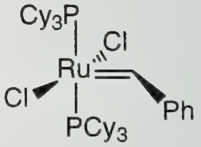
7

Ar = 2,4-diisopropyl benzene



8

Cy = cyclohexyl



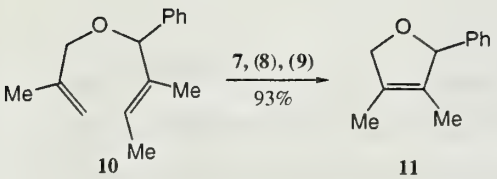
9

Cy = cyclohexyl

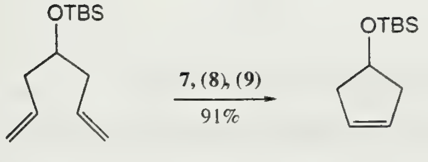
SYNTHETIC UTILITY

Five-, Six-, and Seven-Membered Rings

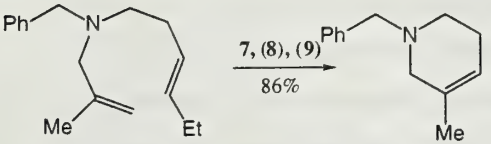
Since the preliminary reports of RCM with **7**, reactions which give 5-, 6-, and 7-membered rings have been thoroughly investigated with catalysts **7**, **8**, and **9**. These catalysts promote RCM reactions to produce carbocycles[9] as well as oxygen and nitrogen heterocycles[5,7b] in good yields. Conversions of **10** to **11** and **12** to **13** are representative examples of the accessible types of ring structures.



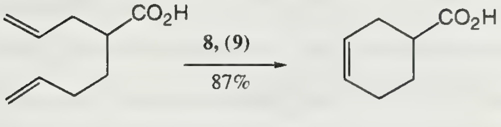
10 **11**



14 **15**



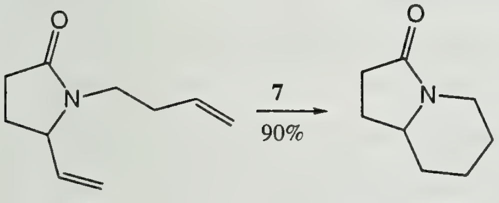
12 **13**



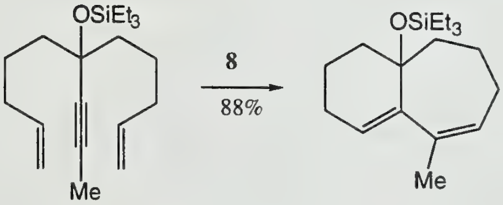
16 **17**

The smooth cyclization of carbocycles with pendant groups has shown catalyst **7** to be compatible with silyl ethers, carboxylic esters, benzyl ethers, and tertiary alcohols.[9] These cyclizations proceed in approximately 85-90 % yields and are exemplified by the conversion of **14** to **15**. The catalyst **8** smoothly catalyzes RCM in the presence of primary and secondary alcohols, carboxylic acids, and aldehydes in good yield. The conversion of **16** to **17** is an illustrative example.[7b] Additional examples of single ring construction are found in the literature.[10]

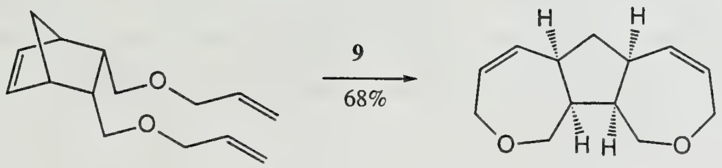
RCM can be used to form fused bicyclic and tricyclic structures. Fused nitrogen heterocycles bearing the nitrogen at one of the bridgeheads have been synthesized, as shown by transformation of **18** to **19**.[11] Double cyclizations of dienynes, illustrated by **20**, demonstrate the ability of the alkyne moiety to relay the metal alkylidene species providing an efficient route to fused bicyclic carbocycles, shown by **21**, in good yields.[12] An interesting variant of the double RCM is the tandem ring opening/ring closing metathesis reaction which demonstrates the capacity of an appropriately placed unsaturated cyclic ring to relay the metal alkylidene species and form tricyclic species as shown by the conversion of **22** to **23**.[13] Numerous examples of bicyclic ring formation similar to those described above have been reported in the literature.[10a, 14]



18 **19**

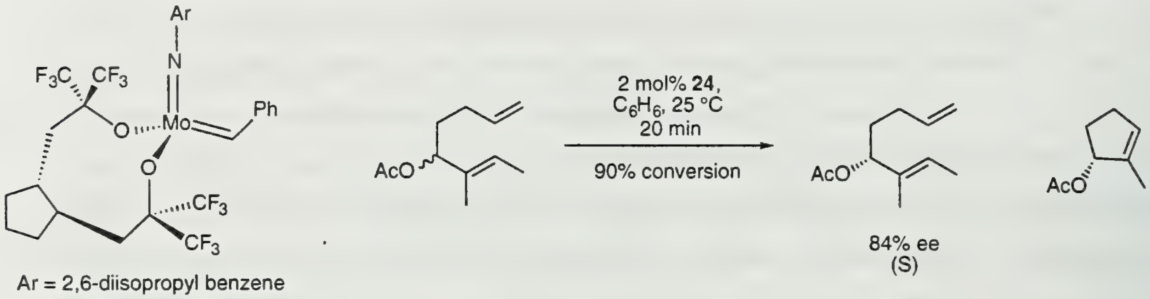


20 **21**



22 **23**

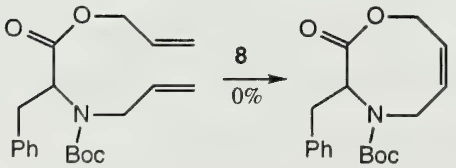
Recent reports from Hoveyda and Grubbs have demonstrated new methods for kinetic resolution of ring closed products. Fujimura and Grubbs reported the first asymmetric ring closing metathesis of dienes using a new chiral molybdenum alkylidene complex **24**.[15] The efficiency of resolution was low as illustrated by transformation of **25** to **27**. At 90% conversion the ee of the unreacted starting material **26** was 84%. Hoveyda *et al.* reported an enantioselective route to small and medium sized rings via ring closing metathesis followed by asymmetric zirconium catalyzed ethylmagnesation. Ring closed products are obtained with up to 99% ee.[16]



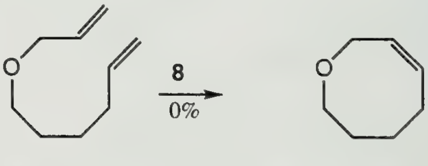
24 **25** **26** **27**

8-Membered Rings

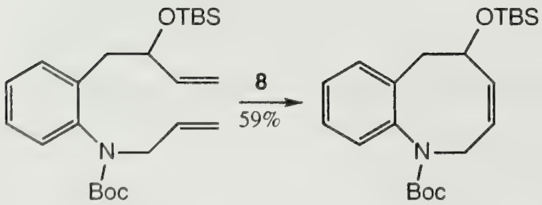
Because RCM is under thermodynamic control, the change in enthalpy in going from reactants to products might outweigh the entropic driving force for cyclization of medium sized rings. Eight membered rings are the only ring sizes in the medium size range which have been investigated. The introduction of conformational constraints on the acyclic diene greatly enhances RCM with **8** for eight membered rings. Diene **28**, with no conformational constraints, fails to undergo RCM. Diene **30**, in which the dienes are constrained by the aromatic ring, cyclizes in 59% yield.[17] The demands for eight membered ring formation can be more subtle. The acyclic ether **32**, gives a mixture of products, whereas the tosyl amide **34**, cyclizes in 68% yield.[18] The necessity for conformational constraints in the formation of eight membered rings has also been demonstrated elsewhere in the literature.[19]



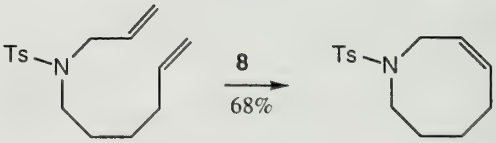
28 **29**



32 **33**



30 **31**

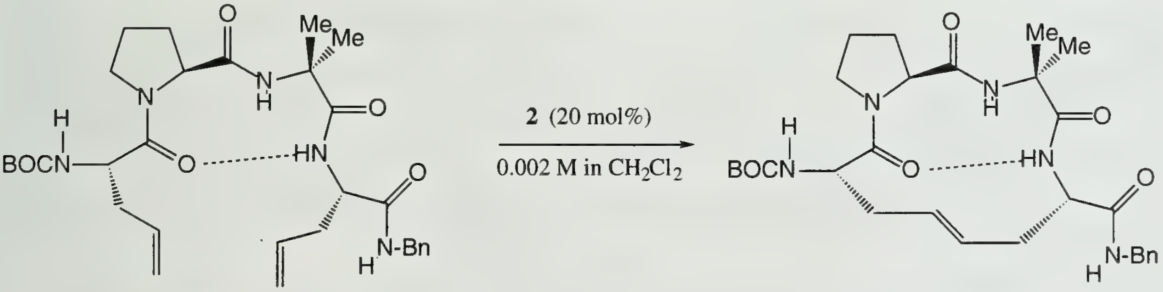


34 **35**

MACROCYCLIC RINGS

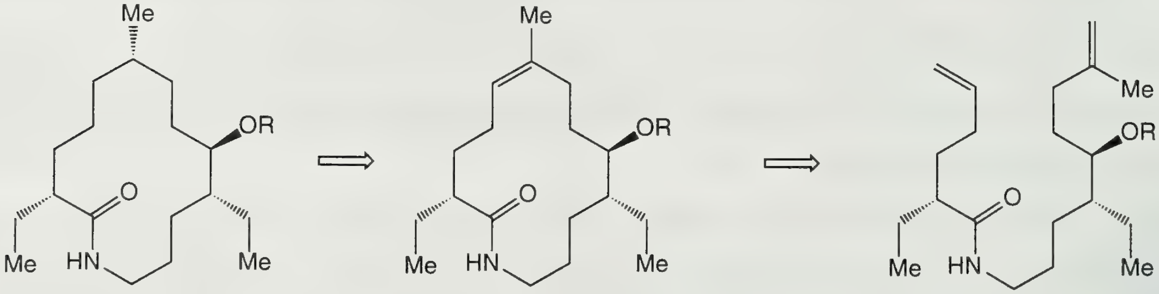
Application of RCM for macrocyclizations has provided closures of rings larger than 12 atoms in four reports. The initial work was performed by the groups of Martin and Pandit. Pandit *et al.* reported the synthesis of the 13-membered ring in Manzamine A using **7**, in 30% yield.[20] The attempted formation of a bicyclo [10.3.0] nitrogen containing macrocycle using **7**, by Martin *et al.* resulted only in dimerization of the starting acyclic diene, with no observable product formation.[11] The acyclic diene was similar in structure to **18** with a 10 carbon terminal olefin extending from the nitrogen.

In efforts to synthesize a peptidic β turn structure, with an alkene in place of the disulfide bridge, Miller and Grubbs found that of all the possible diastereomers of the allyl glycine modified peptide **36**, only one yielded a ring closed product, **37**.[21] In order to determine which diastereomer was cyclized the two most likely candidates, the R,S,R and S,S,S diastereomers of **36** were synthesized in enantiomerically pure form and subjected to cyclization conditions separately. The S,S,S diastereomer was found to cyclize in 60% yield while the R,S,R diastereomer was recovered unchanged. An interesting observation was that the ring closed product was of the same absolute and relative stereochemistry as the original disulfide beta turn structure.



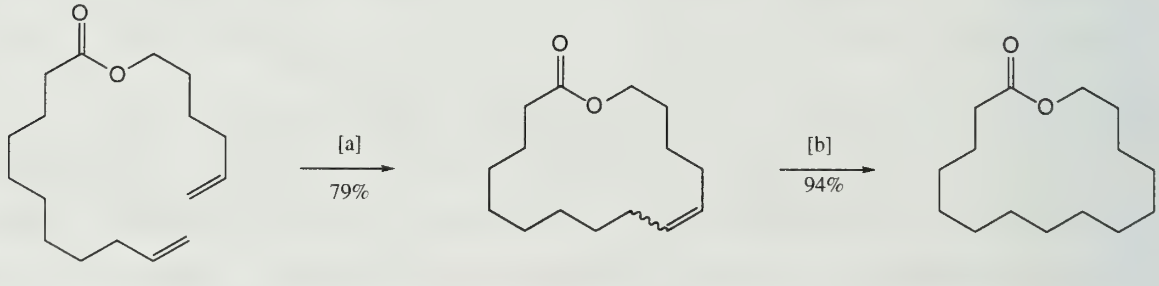
S,S,S - 36 **S,S,S - 37**

Recent work by Hoveyda *et al.* and Furstner and Langeman have shown that acyclic dienes with no conformational predisposition can undergo cyclization in good yields. Compound Sch 38516 is believed to be the same as Fluvirucin B_1 a naturally occurring macrolactam antifungal agent reported to be active against Influenza A.[22] Hoveydas' approach to this class of macrolactams uses RCM for macrocyclization to give the key intermediate **38**.[23] From this intermediate Sch 38516 and other macrolactams of this type are accessible. Hoveyda *et al.* noted that the synthesis of acyclic diene precursor **40**, with the majority of the stereochemistry established prior to the cyclization step, presented a better approach than a macrolactamization approach. Convergent synthesis gave acyclic diene **40** (R = TBS) in good yield. The diene was then cyclized in the presence of 25 mol% of **7**, to give macrocycle **39** in 60% yield (>98% Z). Subsequent hydrogenation of **39** in the presence of 10 mol% Pd/C yielded **38** with greater than 95% diastereoselectivity.



38 **39** **40**

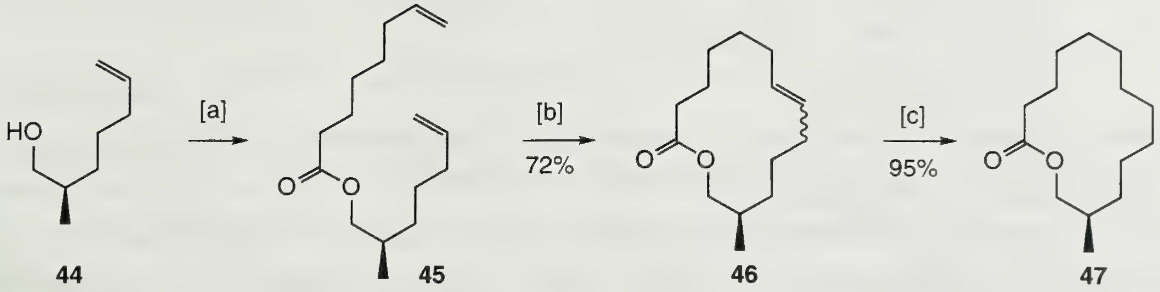
The recent work of Furstner and Langeman may discount the need for conformational constraints and demonstrate that RCM can be a straightforward approach to large ring systems.[24] Starting from available alcohols and carboxylic acids, DCC mediated esterification yielded acyclic diene precursor **41** in high yield. Cyclization in the presence of ruthenium catalyst **8**, followed by hydrogenation gave **43** in 66% overall yield. Analogous 14- and 21-membered macrolides were also synthesized with overall yields ranging from 66% to 72%.



41 **42** **43**

(a) 4 mol% **8**, CH_2Cl_2; (b) H_2 (1 atm), Pd/C.

Also reported was a concise enantioselective synthesis of **47**, (12)-(+)-12-methyl-13-tridecanolide. The asymmetry was introduced in the two steps leading to **44** (>99% ee). Reaction of **44** with 7-octenoyl chloride gave **45** in 85% yield. Cyclization to **46** proceeded in 72% yield (96:4, E:Z) followed by hydrogenation to give **47** (>99% ee). The accessibility of substrates, number of steps, optical purity, and overall yield make RCM an attractive approach toward macrocycle synthesis. The latest results illustrate that conformational predisposition of the reacting olefins is not necessary for macrocyclization.



(a) 7-octenoyl chloride, DMAP, CH_2Cl_2; (b) 4 mol% **2**, CH_2Cl_2; (c) H_2 (1 atm), Pd/C.

CONCLUSION

Ring closing metathesis has been shown to be a viable process in the formation of many sizes of rings. A variety of mono-, bi-, and tri-cyclic small ring systems have been synthesized in synthetically useful yields in the presence of a variety of commonly encountered functional groups. Limited attention has been given to medium size rings and more research needs to be done toward this size of rings. The few reports of RCM in macrocycle synthesis have shown that this is a promising and synthetically useful approach to the synthesis of large ring systems. The advantage that RCM holds for the synthesis of many commercial and biologically active macrocycles is evident. As was demonstrated, previously difficult enantioselective syntheses may be simplified by the establishment of key stereocenters in the acyclic diene prior to the ring closing step. Metal alkylidene catalyzed ring closure can be smoothly performed without disturbance of epimerizable stereocenters.

1. Sehrer, J. C.; Gundiah, S. *J. Sci. Ind.* **1983**, *42*, 250 and ref 1d therein.
2. Grubbs, R. H. In *Comprehensive Organometallic Chemistry*; Wilkinson, G., Stane F. G. A., Abel, E. W., Eds.; Permanganon: Oxford, 1982
3. Grubbs, R. H.; Miller, S. J.; Fu, G. C. *Acc. Chem. Res.* **1995**, *28*, 446-452.
4. (a) Schrock, R. R.; Murzdek, J. S.; Bazan, G. C.; Robbins, J.; DiMare, M.; O'Regan, M. *J. Am. Chem. Soc.* **1990**, *112*, 3875-3886. (b) Schrock, R. R.; Depue, R. T.; Feldman, J.; Yap, K. B.; Yang, D. C.; Davis, W. M.; Park, L.; DiMare, M.; Schofield, M.; Anhaus, J.; Walborsky, E.; Evitt, E.; Kruger, C.; Betz, P. *Organometallics* **1990**, *9*, 2262-2275.
5. (a) Fu, G. C.; Grubbs, R. H. *J. Am. Chem. Soc.* **1992**, *114*, 5426-5427. (b) Fu, G. C.; Grubbs, R. H. *J. Am. Chem. Soc.* **1992**, *114*, 7324-7325.
6. Nguyen, S. T.; Grubbs, R.H.; Ziller, J. W. *J. Am. Chem. Soc.* **1993**, *115*, 9858-9859.
7. (a) Nguyen, S. T.; Johnson, L. K.; Grubbs, R. H. *J. Am. Chem. Soc.* **1992**, *114*, 3974-3975. (b) Fu, G. C.; Nguyen, S. T.; Grubbs, R. H. *J. Am. Chem. Soc.* **1993**, *115*, 9856-9857.
8. Schwab, P.; Grubbs, R. H.; Ziller, J. W. *J. Am. Chem. Soc.* **1996**, *118*, 100-110

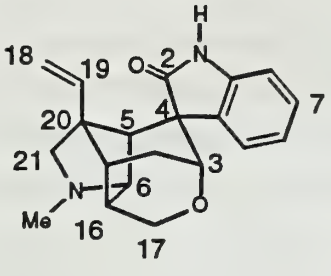
9. Fu, G. C.; Grubbs, R. H. *J. Am. Chem. Soc.* **1993**, *115*, 3800-3801.
10. (a) Fujimura, O.; Fu, G. C.; Grubbs, R. H. *J. Org. Chem.* **1994**, *59*, 4029-4031. (b) Kinoshita, A.; Mori, M. *Synlett.* **1994**, *12*, 1020-1022. (c) Maier, M. E.; Langenbacher, D.; Rebien, F. *Liebigs Ann.* **1995**, 1843-1848. (d) Sita, L. R. *macromolecules* **1995**, *28*, 656-657.
11. Martin, S. F.; Liao, Y.; Chen, H.-J.; Patzel, M.; Ramser, M. N. *Tett. Lett.* **1994**, *35*(33), 6005-6008.
12. (a) Kim, S.-H.; Bowden, N.; Grubbs, R. H. *J. Am. Chem. Soc.* **1994**, *116*, 10801-10802. (b) Kim, S.-H.; Zuercher, W. J.; Bowden, N.; Grubbs, R. H. *J. Org. Chem.* **1996**, *61*, 1073-1081.
13. Zuercher, W. J.; Hashiguchi, M.; Grubbs, R. H. *J. Am. Chem. Soc.* **1996**, *118*, 6634-6640.
14. (a) Schneider, M. F.; Junga, H.; Blechert, S. *Tetrahedron* **1995**, *51*(47), 13003-13014 (b) Holder, S.; Blechert, S. *Synlett.* **1996**, *6*, 505-506.
15. Fujimura, O.; Grubbs, R. H. . *J. Am. Chem. Soc.* **1996**, *118*, 2499-2500.
16. (a) Morken, J. P.; Didiuk, M. T.; Visser, M. S.; Hoveyda, A. H. *J. Am. Chem. Soc.* **1994**, *116*, 3123-3124. (c) Visser, M. S; Heron, N. M.; Didiuk, M. T.; Sagal, J. F.; Hoveyda, A. H. *J. Am. Chem. Soc.* **1996**, *118*, 4291-4298.
17. Miller, S. J.; Kim, S.-H.; Chen, Z.-R.; Grubbs, R. H. *J. Am. Chem. Soc.* **1995**, *117*, 2108-2109.
18. Visser, M. S.; Heron, N. M.; Didiuk, M. T.; Sagal, J. F.; Hoveyda, A. H. *J. Am. Chem. Soc.* **1996**, *118*, 4291-4298.
19. (a) Forbes, M. D. E.; Patton, J. T.; Myers, T. L.; Maynard, H. D.; Smith Jr., D. W.; Schulz, G. R.; Wagener, K. B. . *J. Am. Chem. Soc.* **1992**, *114*, 10978-10980. (b) Martin, S. F.; Liao, Y.; Wong, Y.; Rein, T. *Tett. Lett.* **1994**, *35*(5), 691-694. (c) Martin, S. F.; Wagman, A. S. *Tett. Lett.* **1995**, *36*(8), 1169-1170.
20. Borer, B. C.; Deerenberg, S.; Bieraugel, H.; Pandit, U. K. *Tett. Lett.* **1994**, *35*(19), 3191-3194.
21. Miller, S. J.; Grubbs, R. H. *J. Am. Chem. Soc.* **1995**, *117*, 5855-5856.
22. Hedge, V. R.; Patel, M.; Horan, A.; Gullo, V.; Marquez, J.; Gunnarsson, I.; Gentile, F.; Loebenberg, D.; King, A. *J. Antibiot.* **1992**, *45*, 624-632.
23. Houri, A. F.; Xu, Z.; Cogan, D. A.; Hoveyda, A. H. *J. Am. Chem. Soc.* **1995**, *117*, 2943-2944.
24. Furstner, A.; Langemann, K. *J. Org. Chem.* **1996**, *61*, 3942-3943.

Syntheses of (±)-Gelsemine

Reported by Brian A. Garcia October 3, 1996

I. Introduction

In 1883 crystals of the alkaloid (±)-gelsemine (**1**) were isolated from the plant species *Gelsemium sumpervirens*, commonly known as Yellow Jasmine. The unusual structure of the strong base was finally elucidated 76 years later by X-ray analysis.[1,2] The structural complexity of gelsemine, in combination with the intriguing medicinal usage of the Yellow Jasmine plant, catalyzed a wealth of synthetic endeavors, many unsuccessful.[3-14] However interest in gelsemine again has risen due to three independent total or formal synthesis in 1994, followed by an additional synthesis in 1996.[3,4,15-17]



1

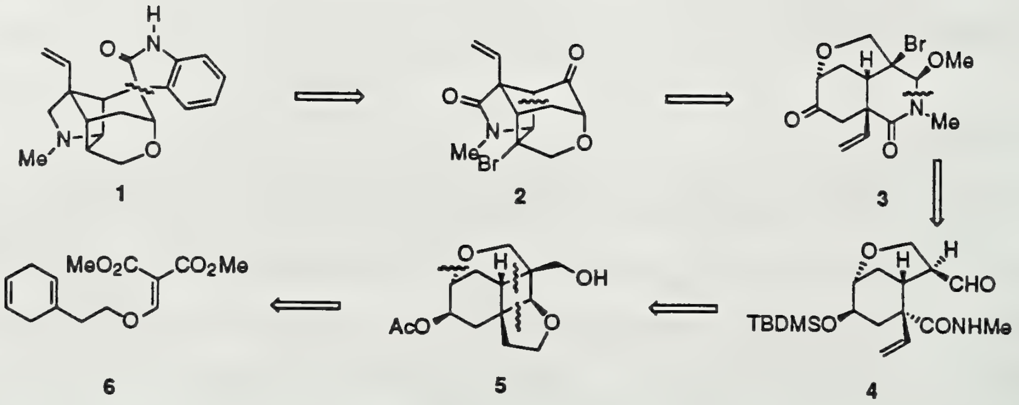
The complexity of gelsemine is evident. The rigid hexacyclic alkaloid is composed of three heterocyclic rings; a pyrrolidine, a pyran, and an oxindole moiety. Additionally, gelsemine contains seven stereogenic centers, two of which are quaternary. One quaternary center (C_{20}) is at the bridgehead of three rings and a vinyl appendage and the other (C_4) is at the junction of the spiro-oxindole and cyclohexane ring. This review will include a discussion and comparison of the four successful routes to date for the total synthesis of (±) Gelsemine or 21-oxo-gelsemine.

II. Retrosynthetic Strategies

The first formal synthesis of gelsemine was by A. Peter Johnson.[3,4] This retrosynthetic approach involves removal of the oxindole moiety in the first disconnection, leaving a ketone **2** (Scheme 1). The next disconnection envisioned was an intramolecular retro-Mannich reaction. The author anticipated creating the rigid tetracyclic molecule using the acidic α-proton and the imine derived from lactam (**3**), leaving three remaining rings. The lactam could be opened in the subsequent step, revealing the aldehyde and amide of highly substituted **4**. This unit was envisioned as being derived from a tricyclic cyclobutane (**5**), which might arise from an intramolecular [2+2] photocycloaddition of triene **7**.

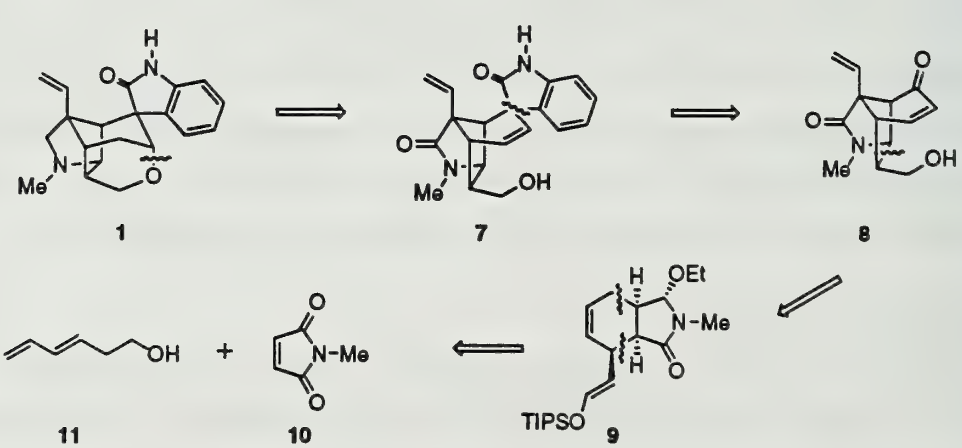
Copyright © 1996 by Brian A. Garcia

Scheme 1



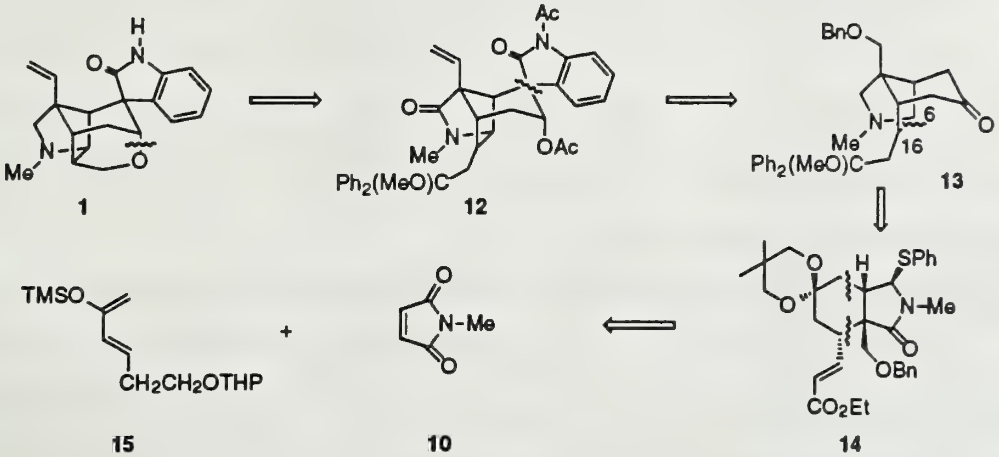
The next reported synthesis of gelsemine, also published in 1994, was by W.N. Speckamp and coworkers (Scheme 2).[15,18-20] In this approach the removal of the tetrahydropyran ring is the first disconnection reducing the complexity and rigidity. The oxindole is removed to give α,β-unsaturated ketone **8** as a key intermediate. Ketone **8** was envisioned to arise from an intramolecular Mannich reaction of enol ether **9** which is easily identified as the product of a endo selective Diels Alder [4+2] cycloaddition between **10** and **11**.

Scheme 2



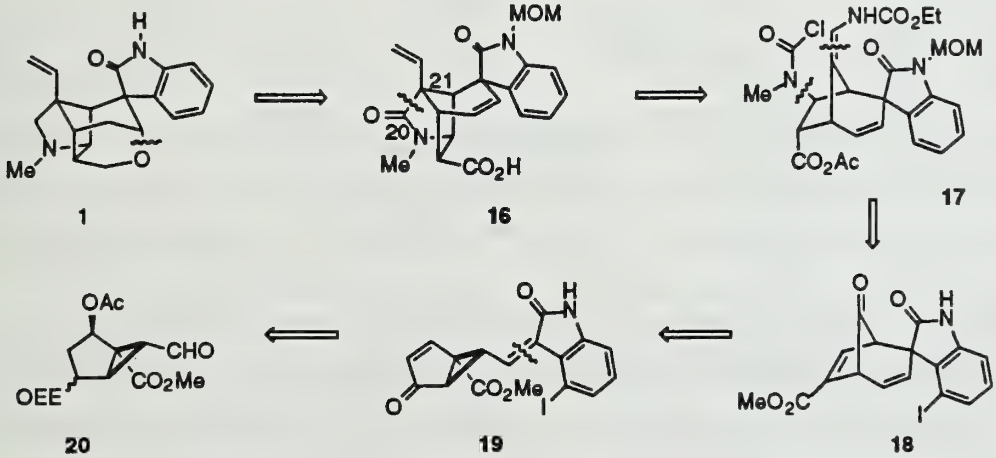
The retrosynthetic analysis of gelsemine by D.J. Hart's group is similar in many respects to the Speckamp strategy, as noted by Hart.[10,11,16] Once again the removal of the tetrahydropyran ring is the first simplification, leaving the protected diol **12**, Scheme 3. The removal of the spiro-oxindole is the second disconnection. Breaking the C_6-C_{16} bond by a retro radical cyclization affords **14**, which is the cycloadduct of diene **15** and maleimide **10**.

Scheme 3



The approach taken by Fukuyama and coworkers is initially similar to those of Hart and Speckamp in that the removal of the tetrahydropyran ring is the first disconnection, providing carboxylic acid **16**.[17] However, at this point the Fukuyama strategy diverges from the previous syntheses. The C20 to C21 bond is cleaved, generating enamide **17**. The subsequent disconnection removes the enamide and chloroformamide, producing **18**. The next set of disconnections, derived from a divinylcyclopropane Cope type rearrangement, leads to divinyl cyclopentane indole **19**. A final disconnection arises from a retro-Knoevenagel condensation.

Figure 4

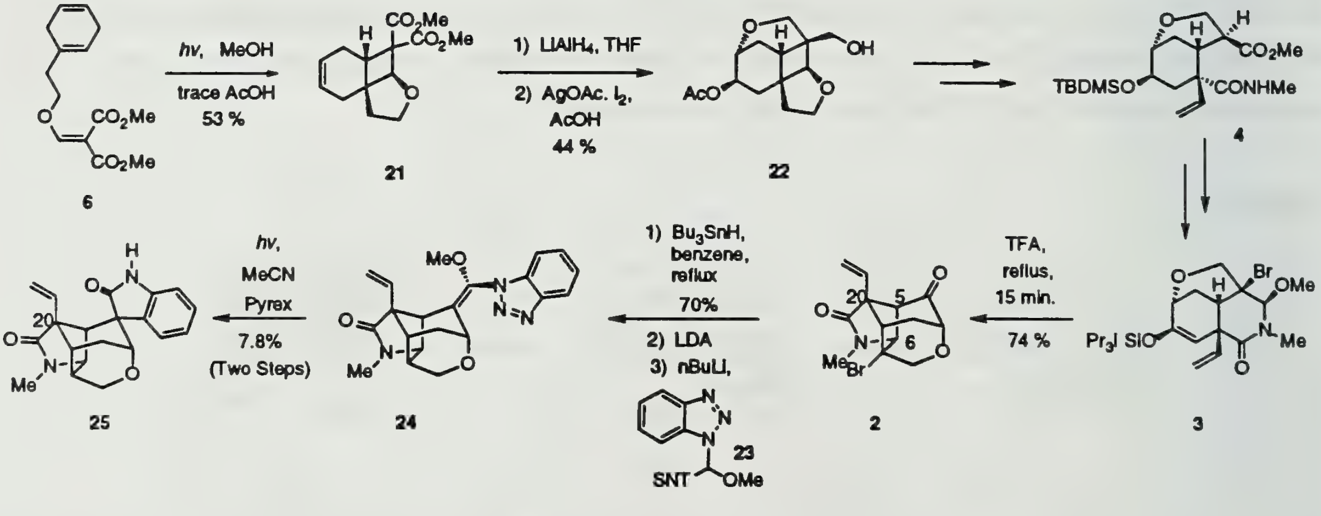


III. Forward Syntheses

In striking contrast to the approaches of Hart and Speckamp, Johnson's route installs the sensitive tetrahydropyran ring very early in the synthesis. An intramolecular [2+2] photocycloaddition of **6**, creates the cyclobutane **21**, Scheme 5. Reduction to the diol, followed by treatment with silver acetate-iodine produced the tetrahydropyran **22**, which interestingly possesses all of the carbon atoms in the core of gelsemine, except for the oxindole and methyl moieties. It also contains the vinyl group, masked as the tetrahydrofuran, with the correct cis orientation with respect to the hydrogen at C_{20}. The cyclobutanone was opened using methylamine to produce amide **4**. Several functional group conversions allowed formation of the key intermediate **3**. Under acid conditions intramolecular attack of the N-acyliminium ion forms the core of gelsemine in 74% yield, a surprising result considering that 5-*endo-trig* cyclizations are usually unfavorable.

The installation of the oxindole utilizes photochemistry developed by Wender for the syntheses of indoles.[4,21] This approach was noted to be quite general, but difficulties were encountered when the ethers were bulky. The addition of the anion of **23** to ketone **2** followed by Peterson olefination gave an E to Z mixture of **24** in a combined yield of 65%. When both olefins were irradiated two cyclized isomers in a 1:2 ratio and combined yield of 36% were produced with 21-oxo-gelsemine as the product. Unfortunately the minor isomer was the desired one, obtained in only 12% yield. This synthesis of 21-oxo-gelsemine required 25 steps and gave an overall yield of 0.27%.

Scheme 5.

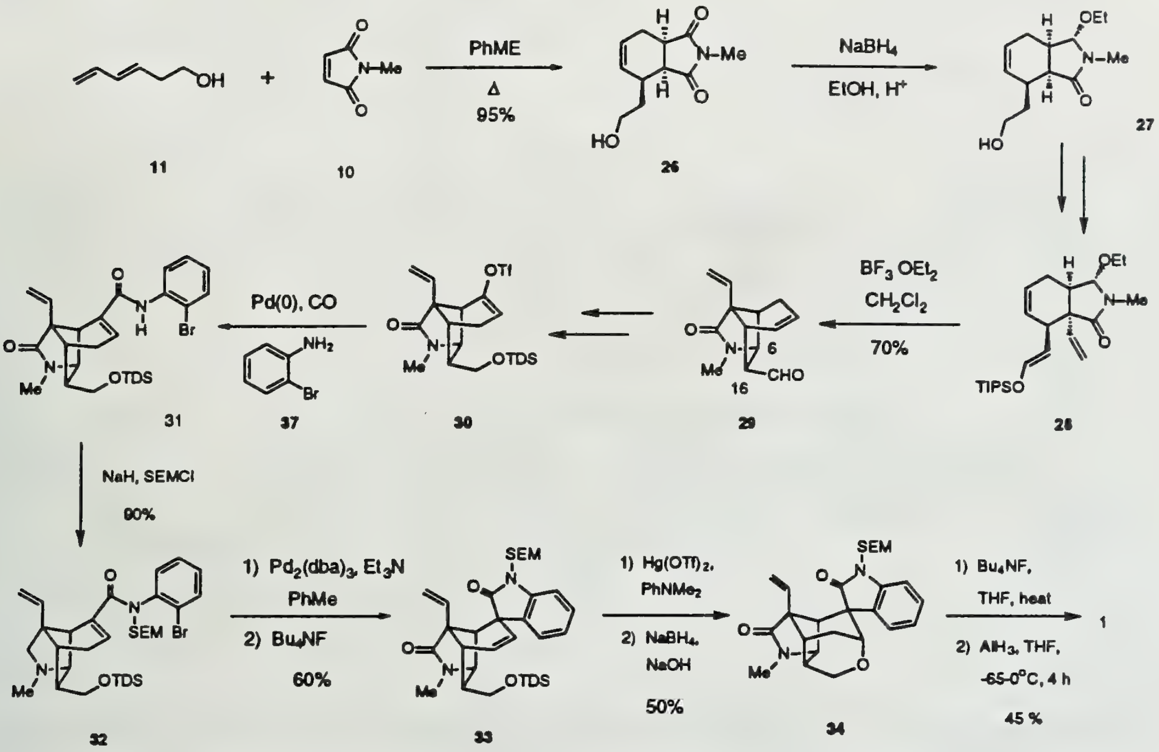


Speckamp's synthesis began with an endo selective Diels-Alder reaction to give imide **26**, (Scheme 6). Chemoselective (3:1) reduction of **26** provided ethoxy lactam **17**. Several more transformations gave **28**, precursor of the key intermediate **29**. Similar to the synthesis by Johnson, an intramolecular N-acyliminium ion capture forms the C_{16}-C_6 bond in **29**. This is in contrast to Johnson's pathway, in which the C_5-C_6 bond is formed.

With the core of gelsemine assembled the two remaining tasks to be completed are the addition of the spiro-oxindole component and closure of the tetrahydropyran ring. After formation of the enol triflate **30**, a palladium-catalyzed carbonylation was performed in the presence of 2-bromoaniline. Ensuing arylation using the ligandless Heck conditions developed by Overman gave the spiro-oxindole

41.[22] The last significant step involved formation of the tetrahydropyran ring using mercury(II) triflate. The entire synthesis involved twenty steps and produced an overall yield of 0.41%

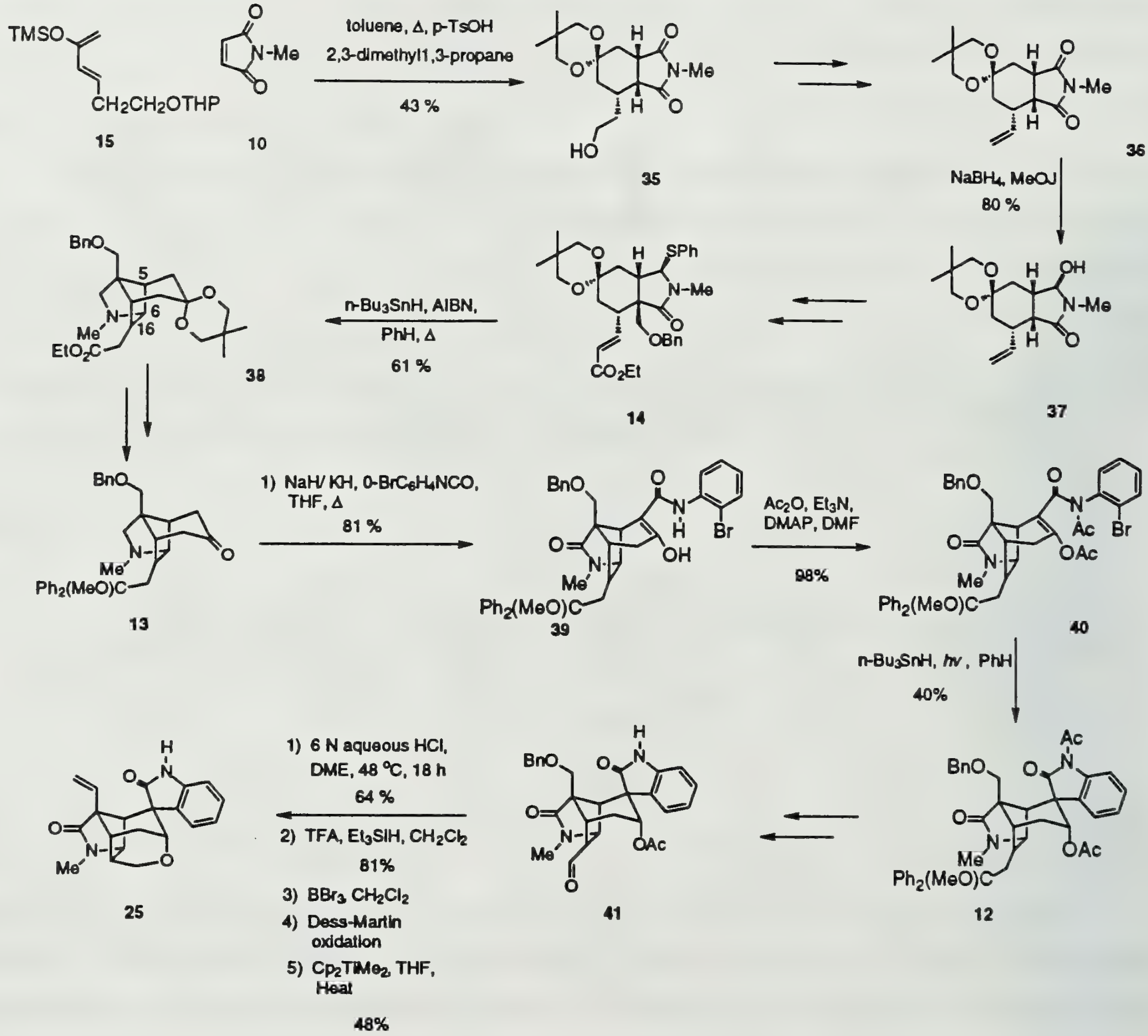
Scheme 6.



Although the retrosynthetic analyses of Hart and Speckamp are apparently similar, in the forward direction these syntheses were quite different. Hart utilized a Diels-Alder reaction of diene **15** and N-methylmaleimide in the presence of 2,3-dimethyl-1,3-propane diol, which afforded the protected ketone **35** (Scheme 7). A moderately selective reduction was performed producing the ethoxy lactam **37**. Further funtional transformations yielded the ether **14,** the precursor to the key intermediate **38**. The radical formed by cleavage of the thiophenoxy bond, via AIBN and Bu_3SnH conditions, attacked the electron deficient alkene of the ester appendage, resulting in formation of the C_{16}-C_5 bond and construction of the gelsemine core **22**.

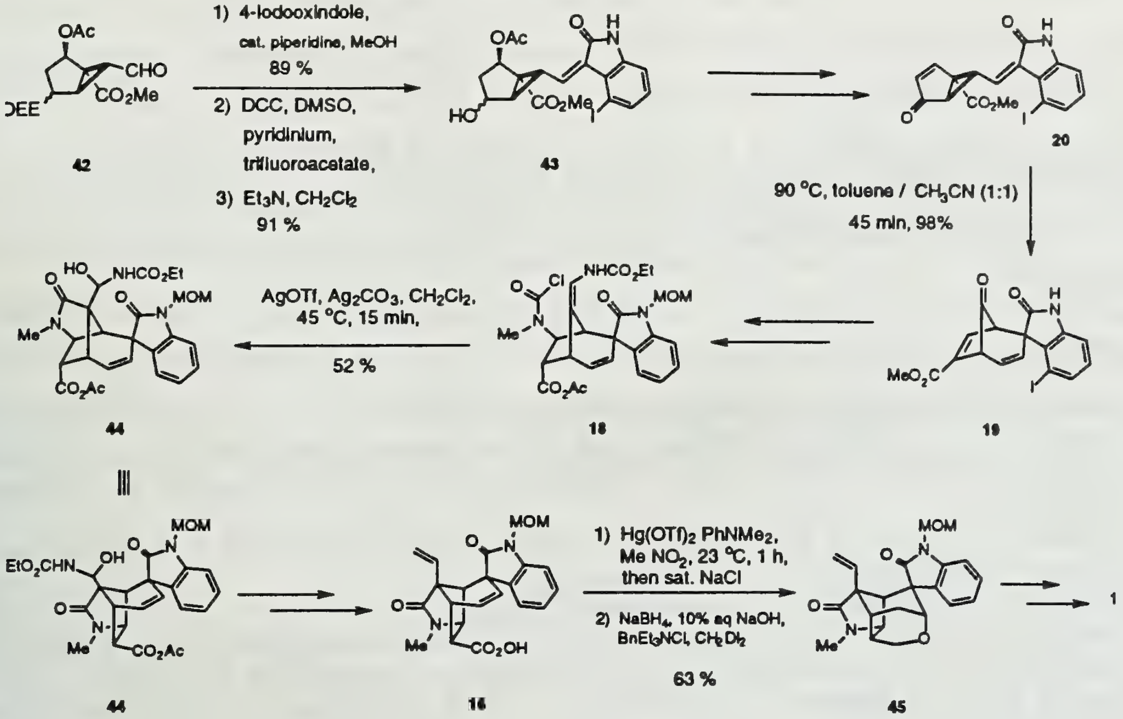
The oxindole and the tetrahydropyran ring were major elements still missing. The installation of the oxindole was accomplished by an acylation of the ketone **13** using NaH and KH in the presence of o-bromophenyl isocyanate, followed by a free radical cyclization to afford **12**. The tetrahydropyran was formed by acid induced attack of the alcohol on the aldehyde side chain. Removal of the secondary alcohol was performed using TFA and Et_3SiH in CH_2Cl_2. The overall yield of 21-oxo-gelsemine was 1.7% in 23 steps.

Scheme 7



The synthesis by Fukuyama is quite different from the syntheses discussed above. This route is based on a divinylcyclopropane-cycloheptadiene rearrangement as the key step, (Scheme 8). The precursor **42** for this step was made in five steps from methyl acetoacetate and sorbic aldehyde in an overall yield of 15.9%. Next the indole was added to the aldehyde side chain via a Knoevenagel condensation. The researchers were able to control the formation of the E and Z isomers by introducing an iodine at the four position of the oxindole prior to condensation. The key thermal rearrangement of **20** produced the desired bicyclic ketone **19**. After several more functional group manipulations enamide **18** underwent a cyclization catalyzed by AgOTf and Ag_2CO_3 to give **44**. The remaining steps entailed formation of the vinyl substituent and closure of the tetrahydropyran ring. The formation of the tetrahydropyran was analogous to that of Speckamp, utilizing mercury (II) triflate in an intramolecular oxymercuration with reductive demercuration. Fukuyama's route towards gelsemine took 32 steps, but still had the highest overall yield of 0.57%, for gelsemine.

Scheme 8



IV. Conclusion

The groups of Overman and Fleming have contributed greatly to the chemistry of gelsemine, but have yet to successfully complete the synthesis. The route attempted by Fleming was based on an intramolecular reaction of an allylsilane with an acyliminium ion, forming the pyrrolidine ring. The tetrahydropyran ring was formed via base catalyzed ether formation. However, Fleming's synthesis was thwarted by an inability to add the oxindole. Overman's approach utilized an aza-Cope Mannich rearrangement to establish the gelsemine core and developed the ligandless palladium catalyzed Heck reaction for installation of the oxindole.[22,23] In the end, however, Overman was unable to form the tetrahydropyran ring.

The successful syntheses of gelsemine and 21-oxo-gelsemine have employed a variety of approaches. The key step in both Speckamp's and Hart's routes formed the C_6-C_{16} bond. Hart utilized an intramolecular radical cyclization and Speckamp used a silyl anion to attack an N-acyliminium ion. Johnson on the other hand formed the C_5-C_6 bond via an intramolecular Mannich in the key step and Fukuyama utilized a Cope rearrangement of a divinylcyclopropane in his key step. Although the retrosynthetic disconnections of Johnson, Speckamp, and Hart are quite similar, they each completed the syntheses using distinctly different pathways.

In general, each of the groups who have attempted the synthesis of this complex molecule make an effort to use their particular methodology. The challenge of this synthesis is demonstrated by the fact that several prominent groups have been unable to synthesize this molecule. In the case of Speckamp he was able to show the synthetic utility of the N-acyliminium ion in intramolecular cyclizations. Hart was able to use his methodology involving radical cyclizations to create both the oxindole and the major portion of the cyclic core. The successful completion of this molecule also required, in some cases, the researchers to utilize methodology developed by others working on gelsemine. For instance Speckamp used Overman's ligandless palladium catalyzed Heck for addition of the oxindole. However, in Overman's attempted synthesis of gelsemine he utilizes N-acyliminium methodology previously developed by Speckamp. In the end successful completion of the goal molecule has allowed the researchers to both showcase their methodology and to finish what has been a long crowded and perhaps continuing race.

References

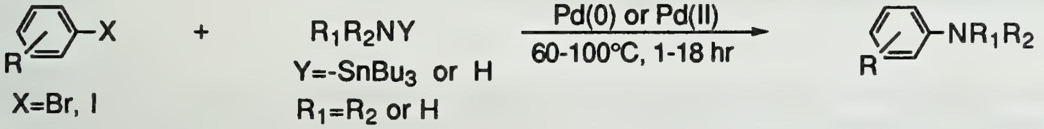
(1) Conroy, H.; Chakrabarti, J. K. *Tetrahedron Lett.* **1959**, 6.
(2) Lovell, F. M.; Pepinsky, R.; Wilson, A. J. C. *Tetrahedron Lett.* **1959**, 1.
(3) Sheikh, Z.; Steel, R.; Tasker, A. S.; Johnson, A. P. *J. Chem. Soc., Chem. Commun.* **1994**, 763-764.
(4) Dutton, J. K.; Steel, R. W.; Tasker, A. S.; Popsavin, V.; Johnson, A. P. *J. Chem. Soc., Chem. Commun* **1994**, 765-766.
(5) Autrey, R. L.; Tahk, F. C. *Tetrahedron* **1967**, 901.
(6) Fleming, I.; Moses, R. C.; Tercel, M.; Ziv, J. *J. Chem. Soc. Perkin Trans. 1* **1991**, 617-626.
(7) Earley, W. G.; Oh, T.; Overman, L. D. *Tetrahedron Lett.* **1988**, *29*, 3785-3788.
(8) Earley, W. G.; Jacobesen, E. J.; Meier, B. P.; Oh, T.; Overman, L. E. *Tetrahedron Lett.* **1988**, *29*, 3781-3784.
(9) Overman, L. E.; Sharp, M. J. *J. Org. Chem.* **1992**, *57*, 1035-1038.
(10) Hart, D. J.; Wu, S. C. *Tetrahedron Lett.* **1991**, *32*, 4099-4102.
(11) Hart, D. J.; Wu, S. C. *Heterocycles* **1993**, *35*, 135-138.
(12) Stork, G.; Krafft, M. E.; Biller, S. A. *Tetrahedron Lett.* **1987**, *28*, 1035-1038.
(13) Jones, K.; Thompson, M.; Wright, C. *J. Chem. Soc., Chem. Commun.* **1986**, 115-116.
(14) Vijn, R. J.; Hiemstra, H.; Kok, J. J.; Knotter, M.; Speckamp, W. N. *Tetrahedron* **1987**, *43*, 5019-5030.
(15) Newcombe, N. J.; Ya, F.; Vijn, R. J.; Hiemstra, H.; Speckamp, W. N. *J.Chem. Soc., Chem. Commun.* **1994**, 767-768.
(16) Kuzmich, D.; Wu, S. C.; Ha, D.-C.; Lee, C.-S.; Ramesh, S.; Atarashi, S.; Choi, J.-K.; Hart, D. J. *J. Am. Chem. Soc.* **1994**, *116*, 6943-6944.
(17) Fukuyama, T.; Gang, L. *J. Am. Chem. Soc.* **1996**, *118*, 7526-7427.
(18) Speckamp, W. N.; Newcombe, N. J.; Hiemstra, H.; Ya, F.; Vijn, R. J.; Koot, W.-J. *Pure Appl. Chem.* **1994**, *66*, 2163-2166.
(19) Hiemstra, H.; Vijn, R. J.; Speckamp, W. N. *J. Org. Chem.* **1988**, *53*, 3882-3884.
(20) Verhaar, M. The Total Synthesis of Gelsemine, University of Amsterdam, 1994.
(21) Wender, P. A.; Cooper, C. B. *Tetrahedron* **1986**, *42*, 2985-2991.
(22) Madin, A.; Overman, L. E. *Tetrahedron Lett.* **1992**, *33*, 4859-4862.
(23) Flann, C. J.; Overman, L. E.; Sarkar, A. K. *Tetrahedron Lett.* **1991**, *32*.

PALLADIUM CATALYZED AROMATIC AMINATIONS: A NEW METHODOLOGY FOR THE CONVERSION OF ARYL BROMIDES AND IODIDES TO ARYL AMINES

Reported by Michael D. Curtis October 7, 1996

INTRODUCTION

Transition metal coupling reactions between aromatic halides and a variety of substrates to make carbon-carbon bonds have proven to be synthetically useful and mechanistically interesting.[1] However, palladium catalyzed carbon-heteroatom bond coupling reactions have been rare. The competitive reaction of ß-hydrogen elimination facilitates decomposition of the reactive palladium intermediate required for the formation of carbon-heteroatom products.[2] New methodologies have been developed to suppress ß-hydrogen elimination and favor the formation of aromatic amines from aryl halides and amines. This review will focus on the palladium catalyzed aromatic aminations of aryl bromides and iodides as shown in equation 1.



(1)

Aryl amines have been commonly prepared indirectly by reduction of aromatic nitro compounds. Traditional methods of direct aryl amine synthesis have required harsh reaction conditions or activated aromatic substrates. The copper catalyzed Ullmann[3,4] condensation of aryl halides with primary[5] and secondary amines[4] typically requires reaction temperatures of 100-300°C and reaction times up to 24 hours. Nucleophilic aromatic substitution of aryl halides with amines requires activated substrates.[6] Unactivated aryl halides can be transformed into aryl amines with sodium and potassium salts of primary and secondary amines via an aryne intermediate.[7] However, the reactions typically afford regioisomeric product mixtures and are limited to aryl halide substrates that are compatible with basic reaction conditions. Nucleophilic substitution of aromatic substrates can also be achieved by a $S_{RN}1$ reaction pathway under very strong reducing conditions which limits the range of substrates that can be utilized.[8]

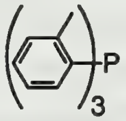
GENERAL REACTION CONDITIONS

Typically 1.0 equiv of aryl halide, 1.5 equiv of amine, and 0.10-2.5 mol% catalyst are stirred

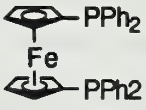
Copyright © 1996 by Michael D. Curtis

at 60-100°C for 1-18 hours in an inert atmosphere of argon. Free amines in the presence of 1.5 equivalents of KO*t*-Bu or $LiN(SiMe_3)_2$, or amino stannanes can be used as the amine source. A palladium(0) intermediate is believed to be the active catalyst and can be prepared and used directly or reduced in situ from a palladium(II) complex.

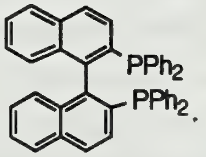
The success of the coupling reaction depends on the choice of the phosphine ligand used. Intermolecular coupling reactions afford aryl amines in high yield with tri-*o*-tolylphosphine(**1**), 1,1'-bis(diphenylphosphino)ferrocene(DPPF) (**2**), and 2,2'-bis(diphenylphosphino)-1,1'-binapthyl(BINAP) (**3**) ligands but not with triphenylphosphine (**4**). Intramolecular cyclizations are successful with both **1** and **4** as ligands.



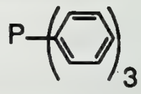
1



2



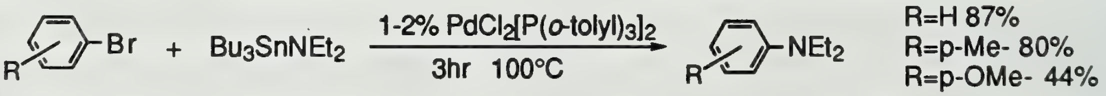
3



4

AROMATIC AMINATION WITH AMINOSTANNANES

The first report of successful palladium catalyzed aromatic aminations was in 1983 by Migita and coworkers[9] in a reaction analogous to the Stille[10] carbon-carbon coupling reaction. When *p*-substituted aryl bromides were treated with *N,N*-diethyl aminotributyltin in the presence of 1-2% $[(o\text{-tolyl})_3P]PdCl_2$, aryl amines were obtained in high yield (eq 2). However, this study was limited to formation of substituted *N,N*-diethyl aniline compounds and little mechanistic information was provided. The instability of other amino stannanes may have contributed to the lack of further development in this area.

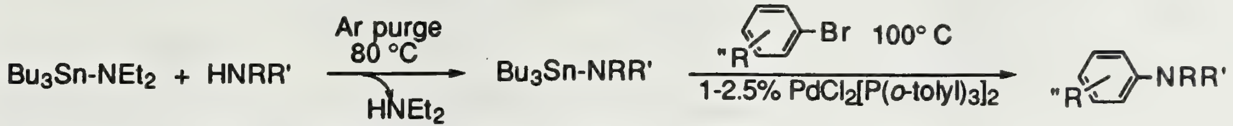


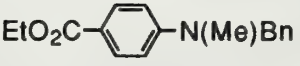
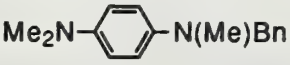
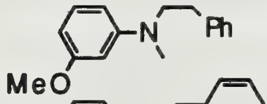
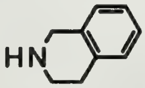
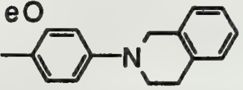
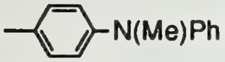
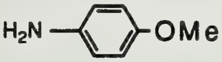
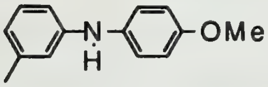
(2)

Following Migita's results, Buchwald and coworkers[11] in 1994 prepared a variety of aniline derivatives through an in situ formation[12] of aminostannanes. Aminostannanes were generated via transamination of *N,N*-diethylamino tributyltin with a less volatile amine. Reaction of aryl bromides, substituted with either electron donating or withdrawing groups, with the aminostannanes afforded a variety of aryl amines with yields ranging between 55-88% depicted in Table 1. Catalyst loadings as low as 1 mol% afforded aryl amines in high yields. The use a primary amines was limited to substituted anilines where as other primary analogs afforded predominantly arene product.

The in situ generation of aminostannanes was extended to intramolecular cyclizations affording five, six, and seven membered nitrogen heterocycles in high yields.[13] Ligands **1** and **4** were effective in the cyclization reaction in contrast to the intermolecular reactions where only **1** was found to promote the coupling reaction (*vide infra*).

Table 1. Palladium Catalyzed Aromatic Aminations with Secondary Aminostannanes



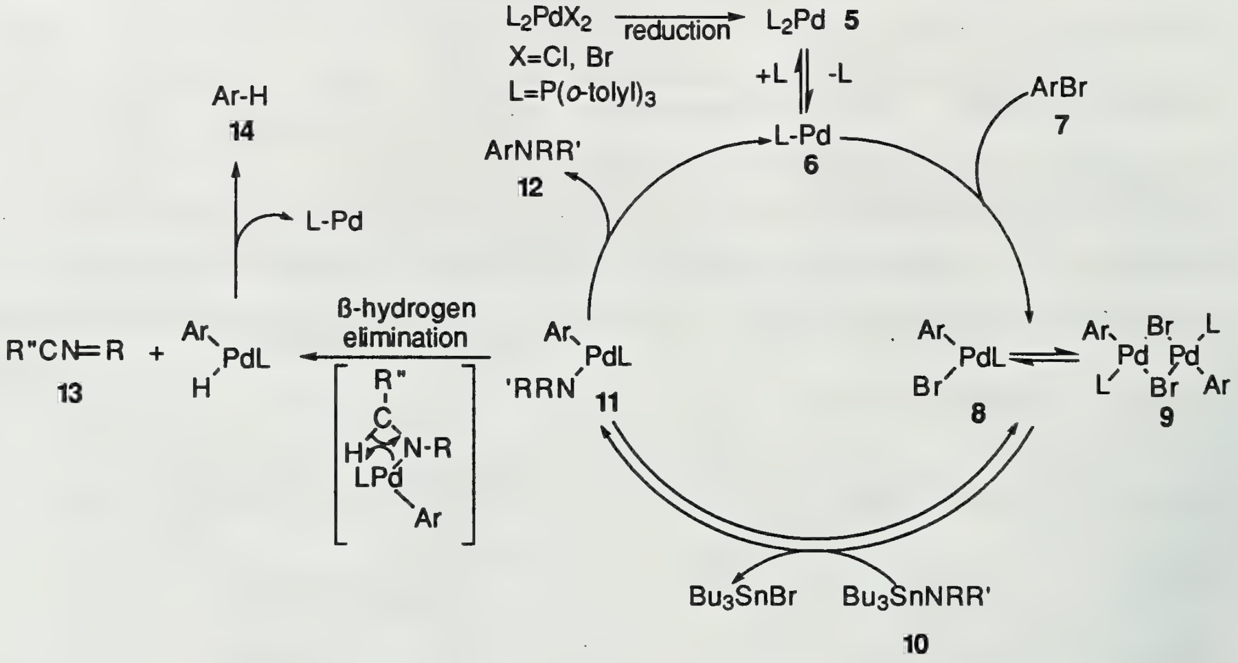
Ar-Br(R")	aminostannane $Bu_3Sn-NRR'$	Product	%yield
p-CO_2Et	HN(Me)Bn		88
p-NMe_2	HN(Me)Bn		81
m-OMe	$HN(Me)CH_2CH_2Ph$		79
p-Me			55
p-Me	HN(Me)Ph		73
m-Me			64

Catalytic Cycle

The mechanism for intermolecular palladium catalyzed amination of aryl bromides **7** with aminostannanes **9** has been investigated by Hartwig and coworkers through extensive kinetic studies and independent syntheses of probable intermediates in the catalytic cycle (Scheme I).[14-19] Kinetic studies suggest that the active catalyst is a monoligated Pd(0) complex **6** formed by ligand dissociation of **5**.[16] Oxidative addition of aryl bromides to **6** produces the dimeric halide-bridged Pd(II) complex **9**.[15] In the presence of excess phosphine, reaction kinetics, as followed by ^{1}H NMR[16] , show the conversion of **6** to **9** to be inverse first order in phosphine ligand concentration under pseudo first order conditions in Pd(0) complex **5**. This result is consistent with oxidative addition to complex **6** where as oxidative addition to complex **5** would be expected to have zero order dependence on phosphine concentration assuming that ligand dissociation is faster than bond breaking and bond making oxidative addition.

Transmetalation[18] of the 14-electron Pd(II) complex **8** formed via reversible dimer cleavage of **9**, with aminostannanes affords Pd(II) amido complex **11**. The reversibility of the reaction made it difficult to determine the reaction orders of the palladium complex. The resting state of the catalyst was determined to be Pd(II) complex **9** which was the main source of palladium complex found in the reaction mixture at short reaction times. Therefore, it was assumed that transmetalation occurs with Pd(II) complex **8**. Transmetalation was inhibited in the presence of Bu_3SnBr which is indicative of a reversible reaction pathway between complexes **8** and **11**. Kinetic experiments also show inhibition by Bu_3SnBr since simple first order dependence in aminostannane was not observed under pseudo first order reaction conditions.

Scheme I

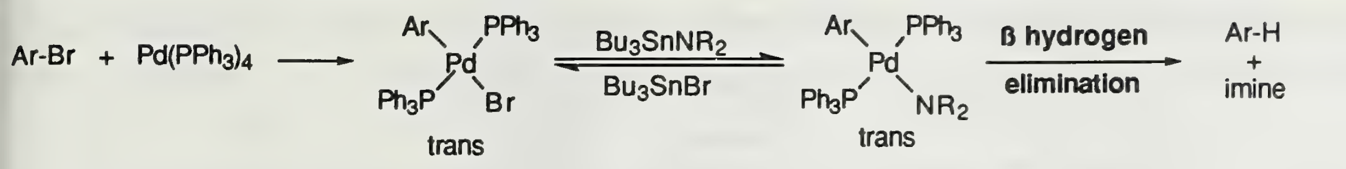


Reductive elimination[17] to give aryl amine **12** was determined to be irreversible since reaction rates and efficiency remained unchanged in the presence of excess aryl amine product. Since the Pd(II) complex **11** could not be isolated, kinetic studies were performed using bis-ligated [$L_2Pd(Ar)NPh_2$]. The reaction exhibited inverse first order dependence on phosphine concentration which is consistent with ligand dissociation from [$L_2Pd(Ar)NPh_2$] to form a complex similar to **11** followed by fast reductive elimination. A zero order reaction dependence on phosphine concentration would be expected for reductive elimination from the bis-ligated [$L_2Pd(Ar)NPh_2$].

The rate determining step in the aminostannane catalytic cycle is a combination of transmetalation and reductive elimination under typical reaction conditions. However, when concentrations of ligand and aminostannane are much greater than aryl halide, the rate determing step shifts to oxidative addition as the major Pd complex in solution is **5**.

Competing ß-hydrogen elimination reaction pathway exists in addition to reductive elimination from Pd(II) complex **11**. Primary aminostannanes were not effective coupling partners due to fast decomposition of Pd(II) complex **11** via this competing reaction pathway (Scheme I) to afford imines and reduced arenes as the major reaction products.

Reactions catalyzed by palladium ligated triphenylphosphine[11,20] were not effective affording imine and arene products. The success of tri-*o*-tolylphosphine palladium complexes has been attributed to its steric bulk which is directly related to the ligand's lability. Tri-*o*-tolylphosphine palladium complexes are considered to facilitate reductive elimination via mono-ligated tri-coordinated Pd amido complexes **11**. Smaller triphenylphosphine ligands form bis-ligated tetra-coordinated palladium complexes in the catalytic cycle which would require a cis geometry for reductive elimination of aryl amines (eq3). Therefore, the experimental results indicate that trans Pd(II)-PPh_3 complexes are initially formed which under go ß-hydrogen elimination faster than isomerization to the cis complex affording imines and arenes.



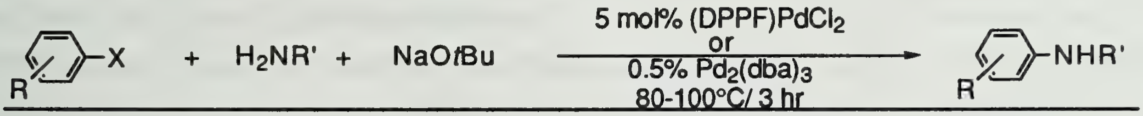
(3)

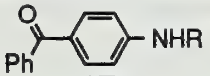
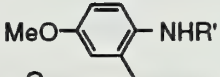
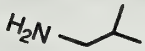
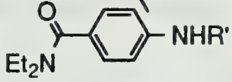
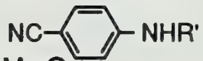
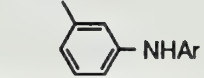
AMINATIONS WITH FREE AMINES

The in situ generation of aminostannanes has clearly extended the methodology of palladium catalyzed aromatic aminations to a large number of secondary amines. However, aminostannanes are toxic and the tin byproducts from the reaction require costly disposal procedures if used in large scale reactions. Hartwig and Buchwald, in independent studies, have shown that both primary[21,22] and secondary amines[23,24] can be effective aminating agents when used in conjunction with an alkoxide or silyl amide base and palladium catalyst.

The chelating ligands, DPPF[22] **2** and BINAP[21] **3**, couple aryl bromides and iodides with primary amines in the presence of an alkoxide base between 80-100°C for three hours (Table 2). The reaction was compatible with electron withdrawing and electron rich aromatic substrates along with alkyl and aryl primary amines as shown in Table 2. Turnover numbers as high as 2000 were observed with BINAP palladium complexes.

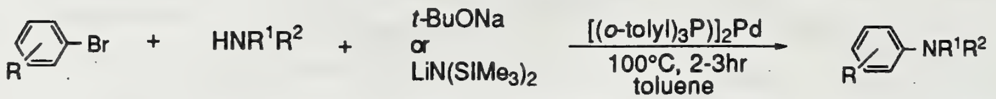
Table 2. Aromatic Amination of Aryl Bromides and Iodides with Primary Amines

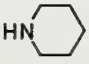
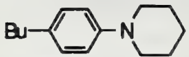
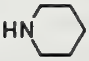
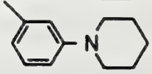
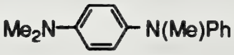
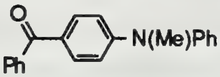
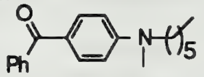
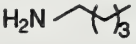
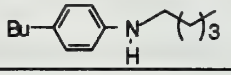


Ar-Br (R=)	Amine	Ligand	Yield	Product
p-C(O)Ph	H_2N-butyl	DPPF	96%	
p-OMe *o*-Me	H_2N-hexyl	BINAP	95%	
p-C(O)NEt$_2$		DPPF	82%	
p-CN	H_2N-butyl	BINAP	93%	
m-OMe X=I	H_2NAr	DPPF	80-96%	

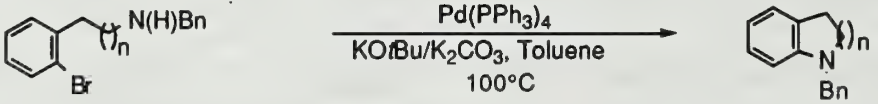
Secondary amines[23,24] were shown to be effective aminating agents in the presence of an alkoxide or silyl amide base. A variety of aryl bromides and iodides were coupled with secondary alkyl and aryl amines affording aryl amines in yields shown (Table 3.). Tri-*o*-tolylphosphine was the most effective palladium complexed ligand with turnover numbers as high as 100, where PPh3 and DPPF ligands were found to be ineffective. Primary amines, were only effective when aniline derivatives, absent of ß-hydrogens, were employed as the aminating agent. Aryl iodides failed to form aryl amines when toluene was used as the solvent; however, in dioxane[25] aryl amines are obtained in good yields.

Table 3. Palladium Catalyzed Aromaic Aminations with Secondary Amines



R-Ar-Br	amine	base	product	%yield
p-Bu		LiN(SiMe$_3$)$_2$ (*t*-BuONa)		89 (89)
m-OMe		LiN(SiMe$_3$)$_2$		94
p-NMe$_2$	HN(Me)Ph	*t*-BuONa		71
p-C(O)Ph	HN(Me)Ph	*t*-BuONa		89
p-C(O)Ph	HN(Me)*n*-hexyl	*t*-BuONa		73
p-Bu		*t*-BuONa		35

Intramolecular reactions are equally effective in the coupling of free amines as demonstrated by Boger[26] and coworkers using stoichiometric Pd[(PPh$_3$)]$_4$ for the cyclization to ß-carboline(84%), a substructure of the anti tumor antibiotic lavendamycin. Buchwald[13] has developed a catalytic variant of Boger's work for the high yielding cyclization of *o*-halo-*n*-benzyl amines (eq 4). The reaction is very similar to the aminostannane cyclization protocol with the advantage of requiring less time and affording higher yields of cyclized product. Cyclizations afforded five, six, and seven membered nitrogen heterocycles with aryl bromides. Triphenylphosphine was the most effective ligand for the intramolecular cyclizations probably due to a chelating effect provided by the proximity of the intramolecular amine nitrogen.[27] The steric bulk of tri-*o*-tolylphosphine ligand likely increases the activation energy of the transition state of both the oxidative addition and chelation steps. These two factors may account for the decreased yield observed with tri-*o*-tolylphosphine ligand relative to the smaller triphenylphosphine ligand.

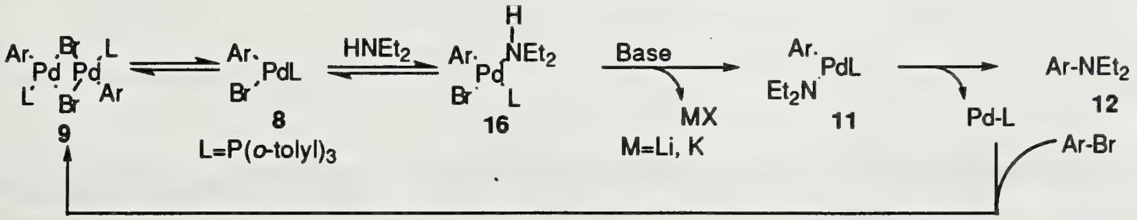


(4)

Attractive as the free amine methodology may appear, the reaction is limited to aryl halide substrates which are devoid of base sensitive functionalities. In such instances, aminostannanes could be a viable alternative.

Catalytic Cycle

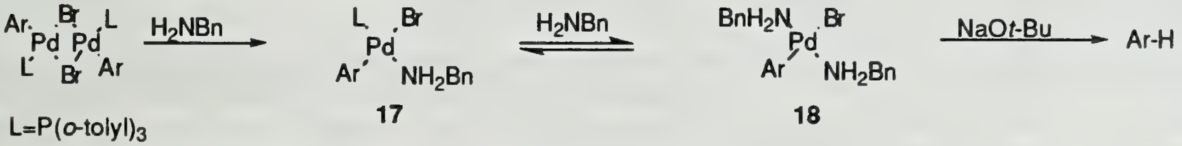
The amination of aryl halides with secondary amines in the presence of base, is believed to proceed much like the aminostannane reaction with ligand **1** (Scheme I). Oxidative addition to the suggested active catalyst **6** affords dimeric Pd(II) complex **9** which is cleaved in the upon addition of an amine to generate the 16-electron Pd(II) amido complex **16** (eq 5). Complex **16** was isolated and characterized.[17] The enhanced acidity of the coordinated amine in Pd(II) complex **16** facilitates deprotonation with alkoxide and silyl amide bases affording aryl amine products at room temperature. ^{1}H and ^{31}P NMR studies suggest the formation of an anionic Pd(III) amido complex when deprotonation is carried out at -70°C. The anionic Pd(III) complex afforded aryl amine products via reductive elimination from a three-coordinated Pd(II) amido complex **11** as the reaction warmed to room temperature.[19] Reductive elimination from a three-coordinate Pd(II) amido complex is supported by the kinetic results discussed for reductive elimination with aminostannanes. The rate limiting step in the reaction with free amines is dependent on the strength of the base employed. When $LiN(SiMe_3)_2$ is used, the major palladium species in solution at short reaction times was PdL_2 indicating oxidative addition to be the rate limiting step. However, when a weaker alkoxide base was employed, a combination of deprotonation and formation of Pd(II) complex **16** was rate limiting.



(5)

The catalytic cycle for the coupling of primary amines with aryl halides is far less developed but presumably proceeds via chelated bis(phosphine) tetra-coordinated Pd complexes.[21] Tetra coordinated palladium-amido complexes were originally thought to suppress product formation however, results recently obtained with chelating DPPF and BINAP ligands contradict this original hypothesis since coupled aryl amine products were obtained in high yields. One possible explanation is that chelating ligands act by "locking" the reactive Pd(II) amido complex into a cis conformation facilitating aryl amine formation via reductive elimination.

A second possibility for the success of DPPF and BINAP ligands may result from the tendency for primary amines to form monomeric bis(amine) Pd(II) complexes **18** via ligand displacement of the mono(amine) Pd(II) complex **17**.[26] These bis(amine) complexes are more prone to undergo ß-hydrogen elimination at a faster rate than reductive elimination in the presence of additional ß-hydrogens (eq 6).



(6)

Additionally, ß-hydrogen elimination has been shown to be facile, via ligand dissociation, from a three coordinate Iridium-amido variant of Vaska's complex,[28] $[(CO)Ir(NPhBn)(PPh_3)]$. Therefore, strong coordinating chelating ligands discourage both the formation of three coordinate Pd(II) amido complexes and bis(amine) Pd(II) complexes both which facilitate formation of arene products via ß-hydrogen elimination.

CONCLUSIONS

Palladium catalyzed aromatic aminations have been shown to be an effective tool for coupling primary and secondary amines to aryl iodides and bromides in high yields. The scope of the reaction is very broad and is applicable to a variety of substituted aromatic substrates including those with base sensitive functionalities. The reaction extends to intramolecular substrates affording easy access to a wide variety of nitrogen heterocycles. The role of the phosphine ligand is critical to the reaction efficiency and further investigation into other potential ligands would lend insight into the factors governing product formation and eventually to optimized reaction conditions.

REFERENCES

(1) Hegedus, L. S. Transition Metals in the Synthesis of Complex Organic Molecules; University Science Books: Mill Valley, CA, 1994.
(2) Bryndza, H. E.; Tam, W. *Chem. Rev.* **1988**, *88*, 1163-1188.
(3) Lindley, J. *Tetrahedron* **1984**, *40*, 1433-1456.
(4) Paine, J. A. *J. Am. Chem. Soc.* **1987**, *109*, 1496-1502.
(5) Matsuoka, M.; Makino, K.; Kitao, T. *Chem Lett* **1979**, 219.
(6) March, J. Advanced Organic Chemistry; 4 ed.; John Wiley & Sons: New York, 1991.
(7) Heaney, H *Chem. Rev.* **1962**, *62*, 81-97.
(8) Bunnett, J. F. *Acc. Chem. Res.* **1978**, *11*, 413-420.
(9) Kosugi, M.; Masayuki, K.; Migita, T. *Chem. Lett.* **1983**, 927-928.
(10) Stille, J. K. *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 508-524.
(11) Guram, A. S.; Buchwald, S. L. *J. Am. Chem. Soc.* **1994**, *116*, 7901-7902.
(12) Jones, K.; Lappert, M. F. *J. Chem. Soc.* **165**, 1944.
(13) Wolfe, J. P.; Rennels, R. A.; Buchwald, S. L. *Tetrehedron* **1996**, *52*, 7525-7546.
(14) Paul, F.; Patt, J.; Hartwig, J. F. *J. Am. Chem. Soc.* **1994**, *116*, 5969-5970.
(15) Paul, F.; Patt, J.; Hartwig, J. F. *Organometallics* **1995**, *14*, 3030-3039.
(16) Hartwig, J. F.; Paul, F. *J. Am. Chem. Soc.* **1995**, *117*, 5373-5374.
(17) Driver, M.; Hartwig, J. F. *J. Am. Chem. Soc.* **1995**, *117*, 4708-4709.
(18) Louie, J.; Hartwig, J.F. *J. Am. Chem. Soc.* **1995**, *117*, 11598-11599.
(19) Louie, J.; Paul, F.; Hartwig, J. F. *Organometallics* **1996**, *15*, 2794-2805.
(20) Hartwig, J. F.; Richards, S.; Barañano, D.; Paul, F. *J. Am. Chem. Soc.* **1996**, *118*, 3626-3633.
(21) Wolfe, J. P.; Wagaw, S.; Buchwald, S. L. *J. Am. Chem. Soc.* **1996**, *118*, 7215-7216.
(22) Driver, M. S.; Hartwig, J. F.; *J. Am. Chem. Soc.* **1996**, *118*, 7217-7218.
(23) Guram, A. S.; Rennels, R. A., Buchwald, S.L. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1348-1350.
(24) Louie, J.; Hartwig, J. F. *Tetrahedron. Lett.* **1995**, *36*, 3609-3612.
(25) Wolfe, J. P.; Buchwald, S. L. *J. Org. Chem.* **1996**, *61*, 1133-1135.
(26) Boger, D. L.; Duff, S. R.; Panek, J. S.; Yasuda, M. *J. Org. Chem.* **1985**, *50*, 5782-5789.
(27) Huheey, J. E.; Keiter, E. A.; Keiter, R. L. Inorganic Chemistry; 4th ed.; HarperCollins: New York, 1993.
(28) Hartwig, J. F. *J. Am. Chem. Soc.* **1996**, *118*, 7010-7011.

RECENT ADVANCES IN STUDIES ON THE POTENTIAL ROLE OF LOW BARRIER HYDROGEN BONDS IN ENZYMATIC CATALYSIS

Reported by Pek Y. Chong October 14, 1996

INTRODUCTION

Low barrier hydrogen bonds have been the focus of a significant amount of debate in recent years because of proposals that they play a critical role in catalysis of proton transfers in enzymes. Many enzymes have a base in the active site which is believed to abstract a proton from the carbon α to the carbonyl of the substrate, to form an enolic intermediate. The rates of these reactions are much greater than would be predicted from consideration of the ΔpK_a between the substrate and the base in aqueous solution. In an attempt to address this kinetic problem, Gerlt and Gassman proposed that the rapid rates can be quantitatively explained by considering the contributions of the thermodynamic barrier, $\Delta G°$, and the intrinsic kinetic barrier, $\Delta G^{\neq}_{int}$, towards the lowering of the activation energy barrier, $\Delta G^{\neq}$. They further proposed that the lowering of $\Delta G^{\neq}_{int}$ is achieved by the prepositioning of the electrophilic catalyst adjacent to the substrate carbonyl group whereas the lowering of $\Delta G°$ is achieved by the stabilization of the enolic intermediate by the formation of very strong hydrogen bonds. These strong hydrogen bonds, which Cleland termed 'low barrier hydrogen bonds' (LBHB)[2] have been proposed to have energies of formation as high as 31 kcal/mol in the gas phase. They are considered to lower the transition state energy by as much as 20 kcal/mol to facilitate difficult reactions, such as enolization of carboxylate groups.[3] Thus, LBHBs could provide the lowering of the activation barrier required to explain the high rates of enzyme catalysis. The proposal that LBHBs are important in enzymatic catalysis has inspired several investigations whose aims were to establish their existence in enzyme active sites and provide a better understanding of their potential role in catalysis.

BACKGROUND

Hydrogen bonds are typically weak interactions, with energies in the range of 3-7 kcal/mol,[4,5] in which a hydrogen is covalently attached to a donor atom and engages in a weak electrostatic interaction with an acceptor atom. The exchange of the proton from one heteroatom to the other is described by a double-well potential energy profile where the barrier between the minima is high, thus confining the

Copyright © 1996 by Pek Y. Chong

proton to the well of the donor. This is illustrated in Fig 1a for the case in which the heteroatoms ar identical atoms. As the hydrogen bond strength to the acceptor atom becomes stronger, the bond length shortens and the parent atoms are drawn in closer. Since the attractive force is canceled out by electron repulsion, second order attractive forces must now come into play in the form of polarization, that induces a dipole in the acceptor atom, and delocalization of charge.[5] These provide the extra energy for strong and very strong hydrogen bonds. A consequence of this is the lowering of the barrier separating the wells (Fig 1b). In very strong hydrogen bonds (Fig 1c), the barrier is very low, or non-existant.[5] In this case, the H is equally and essentially covalently bonded to both heteroatoms.[3,15] Based on crystal structure correlation methods, Gilli *et al.* have confirmed the hypothesis that the degree of covalency in the O-H···O bond increases continuously with the shortening of the bond. They have also suggested that very short hydrogen bonds are to be considered totally delocalized three-center-four-electron covalent bonds.[6] It necessarily follows from this concept, that the symmetry of the hydrogen bond, where the parent atoms are the same, increases as it gets shorter. Support for this corollary is also provided by Gilli *et al.* who showed by, crystal structure correlation methods, that the transformation of a disymmetrical O-H···O electrostatic interaction to a covalent and symmetrical O-H-O bond occurs with decreasing hydrogen bond length.[6] The limiting case of a strong hydrogen bond is one where the H is positioned midway between the two partners, a condition which is met when the donor and acceptor have equal pK_as. Consequently, a key requirement for LBHB stabilization is the close matching of pK_a of the two donors.[2,3,7,8]

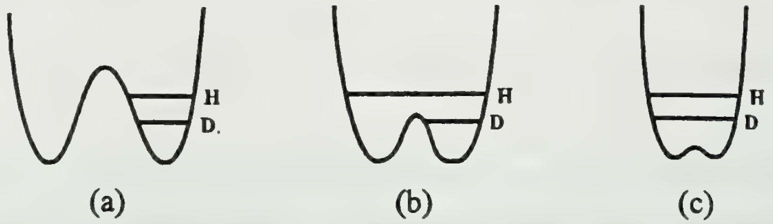


Fig 1. Potential energy wells for hydrogen bonds. (a) double-well (weak) hydrogen bond (b) low-barrier (strong) hydrogen bond (c) single-well (very strong) hydrogen bond. The horizontal lines represent the lowest vibrational energy for hydrogen (upper) and deuterium (lower). [4,5]

Until recently, information on hydrogen bond symmetry was accessible only through gas phase microwave spectroscopy or solid state X-ray or neutron spectroscopy. Classical assignments in solution have used IR spectroscopy, which is difficult to apply to water and relies on empirical correlations to other hydrogen bonds that are thought to be symmetric.[7a] Perrin has recently used the nuclear magnetic resonance method of isotopic perturbation to determine the symmetry of hydrogen bonds in solution to distinguish between single- and double-well potential profiles in intramolecularly hydrogen bonded complexes. The symmetry of the bond was also found to be strongly solvent dependent. The isotope shift of hydrogen maleate anion measured in THF, DMSO, acetonitrile and water showed that the anion has a symmetric hydrogen bond in the least polar solvent, THF.[7] However, the hydrogen bond became more unsymmetrical as solvent polarity increased. Perrin postulated that the dissymmetry was due to the disorder of water which made it improbable for the carboxyl groups to be identically solvated thereby affecting the ΔpK_a.[7] Perrin's studies demonstrate the importance of the local environment in determining the symmetry and therefore the strength of hydrogen bonds.[7] Indeed, Frey has stated that LBHBs cannot be formed in water.[8] The operation of LBHBs in the active site of an enzyme where hydrogen bonding solvent is excluded is consistent with these experiments.

NATURE OF THE LBHB

LBHBs are characterized by short donor-acceptor atomic distances (< 2.5 Å), very broad, low-frequency IR bands, strongly deshielded ^{1}H NMR chemical shifts of the proton, and very low ^{1}H, ^{2}H isotope fractionation factors (φ).[2,5,8,9] Although there is evidence, from these criteria, for the formation of LBHBs in solution and in the solid state, quantitative information on the strength of these bonds is available only from gas phase measurements and computational models.[5,6] Guthrie has pointed out that stabilization effects are much larger in the gas phase than in solution due to the need of the high energy ions in the gas phase for stabilization.[4] This was confirmed by Drueckhammer *et al.* who found that the estimated hydrogen bond energies in enzyme active sites and in solution are much smaller than when observed in the gas phase.[10-12] Therefore, one cannot rely on gas phase stabilization energies to predict those in solution or in the catalytic site.[4,20] In addition, observation of a short or symmetric bond in the solid state cannot be used to estimate its strength in solution, although this correlation is often taken for granted. Drueckhammer *et al.*, in their studies on enzyme-inhibitor complexes, found no direct correlation between hydrogen bond length and its strength.[10-12,14] They caution against any attempts to correlate the two. *Ab initio* calculations by Scheiner *et al.* have shown no additional strengthening from the shortening of a strong hydrogen bond.[13] Furthermore, comparison of isotope shifts of LBHBs in crystalline and aqueous media by Perrin showed that the symmetry seen in crystals is broken in aqueous solution. This, he states, is a "remarkably simple counterexample to the prevailing hope that a crystal structure describes the solution structure."[7a] There is therefore some contention regarding whether the hydrogen bond length can be taken as proof of the existence of a LBHB.

PROPOSED ROLES OF LBHB IN ENZYMATIC CATALYSIS

Studies on the possible roles of LBHBs in enzymatic catalysis have relied primarily on spectroscopic data. The least ambiguous parameter for characterizing LBHBs is considered to be the NMR chemical shift of the participating proton which has been found to range between 16-20 ppm. Frey *et al.* have assigned a signal at 18.3 ppm in the NMR of chymotrypsin at pH 3.5 to a proton involved in a LBHB between the histidine and aspartate residues in the active site (shown in structure 2 in Fig. 2).[15] The assignment is based on coupling of the proton to ^{15}N in the protease. The deuterium isotope effect on the chemical shift was 1.0 ppm ± 0.4 ppm which falls in the range of that expected for LBHBs.[5] From these results, Frey *et al.* proposed that formation of a LBHB between Asp^{102} and a N of His^{57} facilitates the nucleophilic attack of Ser^{195} on the substrate carbonyl group, by deprotonation. The LBHB is proposed to stabilize the transition state by 12-24 kcal/mol, thereby reducing activation energy and enhancing rate of reaction. The proposed mechanism is further supported by the observation that N-methylation of His^{57} decreases acylation rate by $2x10^5$.[15]

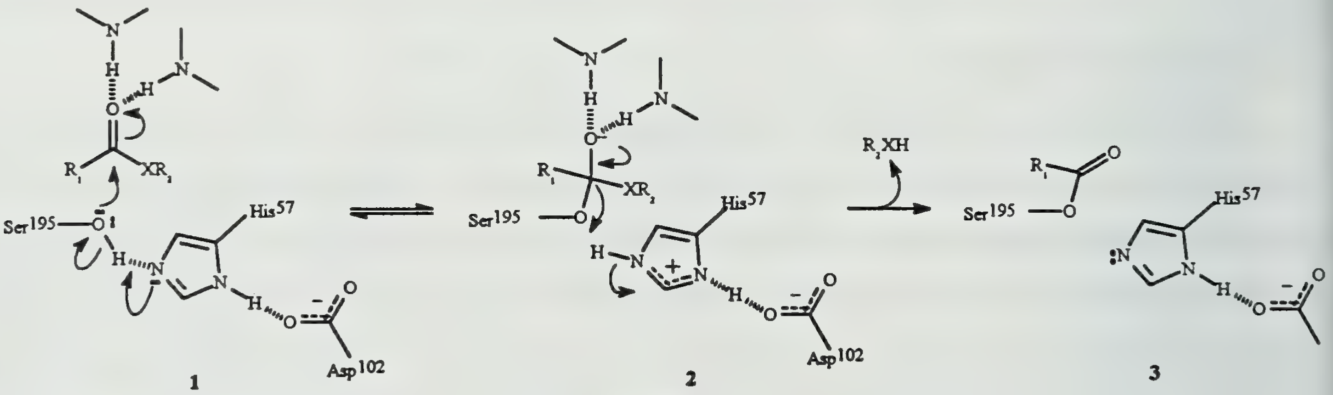


Fig 2. The proposed LBHB-facilitated general base mechanism for the acylation of chymotrpsin. Stabilization of the catalytic triad by the LBHB lowers the activation energy for its formation.[8,15]

Further studies by Frey *et al.* employed NMR and FTIR spectroscopy to study a series of intermolecular complexes formed between carboxylic acids and 1-methylimidazole (which is a first approximation of the model proposed for the catalytic triad).[8] FTIR data indicate that the hydrogen bonds are strong and are of three types, as shown on Fig. 3 : neutral complexes with the weaker acids (I), ionic complexes of stronger acids (II), and complexes described by III which exhibit values of antisymmetric C=O stretching frequencies that are intermediate between those of I and II. Complex III corresponds to a species in which the C=O bond order is between 1.5 and 2. The presence of LBHBs is invoked to explain the spectroscopic data. The results from these studies are compatible with strong hydrogen bonding in the catalytic triad of serine proteases.[8]

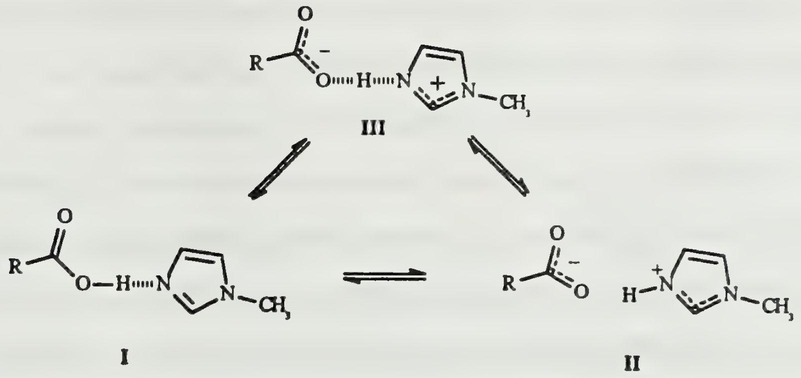


Fig 3. Frey's NMR and FTIR studies on complexes between carboxylic acids and 1-methylimidazole show the presence of three different complexes, (I) neutral, (II) ionic and (III) low barrier hydrogen bonded complexes.[8]

LBHBs have been identified or infered in several other enzymes. Dunn *et al.* recently provided evidence of a LBHB in the tryptophan synthase catalytic mechanism based on the unusual fractionation factor of 0.62.[14] Tong and Davis proposed that three LBHBs serve to anchor the PLP cofactor in the active site of 2-amino-3-ketobutyrate-CoA ligase, based on ^{1}H NMR chemical shifts and assignments of resonances, established by NOE.[16] The finding that the three low field ^{1}H resonances disappeared, when the PLP cofactor was removed, fully supports their proposal. ^{1}H NMR data suggest the existence of similar LBHBs in several other PLP enzymes. [17] Recently, following many studies on the catalytic mechanism of Δ^5-3-ketosteroid isomerase[18], Zhao *et al.* has provided direct NMR evidence for the participation of a LBHB.[19] Their conclusions are based on the observation of a highly deshielded proton with a 3-fold preference for protium over deuterium ($\varphi = 0.34$) which is consistent with the existence of a LBHB between Tyr^{14} and the dienolic intermediate of the isomerase. When an analog of the intermediate (DHE) was replaced with 4-fluoroestradiol, which increases the ΔpK_a to Tyr^{14}, the deshielding effect decreased. They suggest that the LBHB is a significant contributor to the stabilization of the intermediate and thus the catalytic power of the enzyme.

Assignments of LBHBs in proteins have been associated primarily with the catalytic site apparatus and/or coenzyme interactions with the site.[14-19,21] However, these studies can only support the LBHB hypothesis; they cannot discount other explanations. The roles played by LBHBs in catalysis are not clear.[15] Warshel *et al.* argue that the evidence for short bond lengths of LBHBs in enzymes does not imply an unusually strong bond nor does the presence of a LBHB mean that it has an important role in catalysis.[20] The studies to date have shown that there is a class of hydrogen bonds that show unusually low deuterium isotope fractionation factors, downfield shifted ^{1}H-NMR signatures, and IR spectra with broad continuum absorption. If these LBHBs do not play a role in enzyme catalysis, then it is an unusual coincidence that they have all been associated with the catalytic apparatus of the enzymes.

The lack of a structure-function relationship has led several research groups to question the relevance of these observations to the strength of the hydrogen bond and its role in enzyme catalysis.[4] Although the observation of spectroscopic signatures have been used to identify LBHBs, none of these measurements can directly assess the energetics of the transition from a weak hydrogen bond to a strong one. The literature of experimentally determined hydrogen bond energies is rather sparse[13,5] and measurements of the energetics of LBHBs in transition states are not possible.[14]

ENERGETICS OF LBHB FORMATION

The lack of evidence for the strength and role of LBHBs has led to much debate, the point of contention being whether these LBHBs provide 10-20 kcal/mol stabilization energy[3] in the active site. Guthrie points out that there is no experimental evidence for the existence of very strong hydrogen bonds in solution.[4] Drueckhammer *et al.* have developed a method to study the relative strengths of normal and LBHBs in solution by examining cis-trans isomerization equilibria of unsaturated diacids and their analogous monoacids.[11] The isomerizations of maleic/fumaric and citraconic/mesaconic acids were studied (Fig. 5). An intramolecular LBHB was shown to exist in the cis isomer of maleic acid by Perrin.[7] Comparison of the equilibrium constants, K_{eq}(anion) and K_{eq}(diacid), allowed an assessment of the relative abilities of a LBHB and a normal hydrogen bond to stabilize the cis isomers. The difference in these equilibrium constants gave a measure of the additional stabilization conferred upon the anion cis isomer by a LBHB. Their data provide good evidence for a 4.4 kcal/mol greater strength of the LBHB in DMSO relative to the pK$_a$-mismatched hydrogen bond (which was shown in Perrin's work to have a double-well

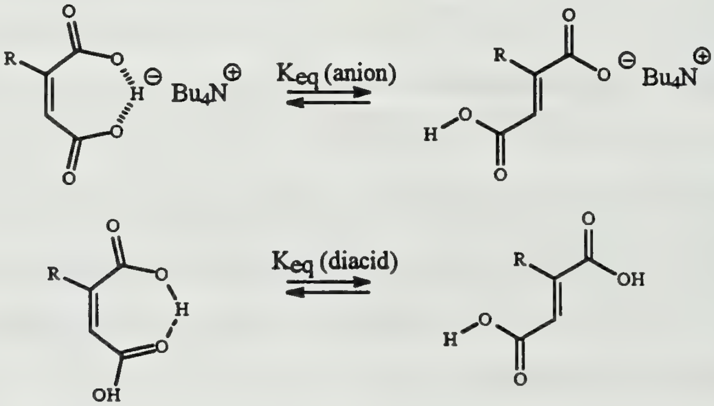


Fig 5. Intramolecular hydrogen bonding in the cis isomers of the maleic/fumaric and citraconic/mesaconic acid systems.[11]

potential energy profile). Drueckhammer *et al.* obtained an even smaller figure of ~2 kcal/mol difference in binding energy between a carboxylate and an amide analog of an inhibitor.[10] Both values fall short of the expected 10-20 kcal/mol stabilization energy of LBHBs.

Recently, Herschlag *et al.* studied the energetics of hydrogen bonds in organic solvents by examining hydrogen bond formation constants as a function of ΔpK_a.[22] They found that additional stabilization from formation of a LBHB from a weak hydrogen bond was only 7.9 kcal/mol for the salicylic acid monoanion in DMSO[27], 4-5 kcal/mol for phthalate monoanion derivatives in DMSO, and 2.7-6 kcal/mol for phenol-phenolate derivatives in THF. This study supports Drueckhammer's finding that the stabilization energy of LBHBs relative to a normal hydrogen bond is substantially less than 10-20 kcal/mol. Scheiner *et al.* reached the same conclusion using *ab initio* calculations.[13] Energetics studies are in general agreement that the additional stabilization of the LBHBs in solution is not sufficient to account for the 10-20 kcal/mol lowering of the activation barrier needed to explain the rates of enzyme catalysis.

Herschlag *et al.* have examined the mechanism of catalysis by ribonuclease, which has been proposed by Gerlt and Gassman to stabilize a phosphorane intermediate via LBHBs. They have concluded that short, strong hydrogen bonds do not contribute substantially to RNase A catalysis.[25] Their conclusions are based on the observation that when they substituted either of the non-bridging phosphoryl oxygen atoms in 2'3' cyclic UMP by sulfur, which is a much poorer hydrogen bond acceptor than oxygen, there was only a 5 fold reduction in reaction rate. They conclude that this is too modest of a thio-effect for strong hydrogen bonds to be the major contributors to catalysis by RNase A.

A limited number of attempts have been made to provide an alternative explanation for the rates of enzymatic catalysis. Herschlag *et al.* and Warshel *et al.* have suggested that electrostatic stabilization of the transition state is sufficient to account for the major part of the catalytic effects of enzyme systems.[20] Cleland and Kreevoy responded by pointing out that this is misleading because the binding energy is based on the wave functions of the interacting system and not on the isolated reactants, or on a particular partitioning scheme.[26] Although Herschlag *et al.* do not believe that LBHBs can account for the rates of enzymatic catalysis, they propose that the energetic effects of multiple hydrogen bonds, each of moderate strength, can be combined to make a significant contribution to enzymatic catalysis. They also suggest that the prepositioning of hydrogen bonding groups in an active site, to minimize entropic costs, can contribute to enzymatic catalysis.[27] This, however, has already been proposed by Gerlt and Gassman who stated that this prepositioning reduces the activation barrier by lowering the intrinsic kinetic barrier, $\Delta G^{\neq}_{int}$.[1] In addition, it is essential to note that the original proposal of Gerlt and Gassman did not state that the lowering of the activation barrier could be explained solely by the formation of strong hydrogen bonds. Their proposal of a contribution by a lowering of $\Delta G^{\neq}_{\text{int}}$ has largely been ignored.

CONCLUSION

The discovery of LBHBs, as defined by a short donor-acceptor atomic distances (< 2.5 Å), very broad, low-frequency IR bands, strongly deshielded ^{1}H NMR chemical shifts of the proton, and low ^{1}H, ^{2}H isotope fractionation factors (φ), in the active sites of enzymes implies that they have a role in enzyme catalysis. However, agreement on these roles has not been forthcoming. Recent energetics studies imply that short, strong hydrogen bonds that stabilize the transition state cannot alone account for the rates of enzyme catalysis. It is experimentally difficult to establish the significance of the role that LBHBs play in enzymatic catalysis, due to the scarcity of good solution models. It is due to the scarcity of relevant experimental data that the potential role of LBHBs in enzyme catalysis continues to be debated.

1) a) Gerlt, J. A.; Gassman, P. G. *J. Am. Chem. Soc.* **1993**, *115*, 11552. b) Gerlt, J. A.; Gassman, P. G. *Biochemistry* **1993**, *32*, 11043.
2) Cleland, W. W. *Biochemistry* **1992**, *31*, 317.
3) Cleland, W. W.; Kreevoy, M. M. *Science* **1994**, *264*, 1887.
4) Guthrie, J. P. *Chem. Biol.* **1996**, *3*, 163.
5) Hibbert, F.; Emsley, J. *Adv. Phys. Org. Chem.* **1990**, *26*, 255.
6) Gilli, G.; Ferretti, V.; Bertolasi, V.; Gilli, P. *J. Am Chem. Soc.* **1994**, *116*, 909.
7) a) Perrin, C. L. *Science* **1994**, *266*, 1665. b) Perrin, C. L.; Thoburn, J. D. *J. Am. Chem. Soc.* **1992**, *114*, 8559.
8) Frey, P. A.; Cassidy, C. S.; Tobin, J. B.; Whitt, S. A. *Biochemistry* **1995**, *34*, 6919.
9) Kreevoy, M. M.; Liang, T. M. *J. Am. Chem. Soc.* **1980**, *102*, 3315.
10) Drueckhammer, D. G.; Martin, D. P.; Remington, S. J.; Usher, K. C. *Biochemistry* **1994**, *33*, 7753.
11) Drueckhammer, D. G.; Schwartz, B. *J. Am Chem. Soc.* **1995**, *117*, 11902.
12) Drueckhammer, D. G.; Schwartz, B.; Usher, K. C.; Remington, S. J. *Biochemistry* **1995**, *34*, 15459.
13) Scheiner, S.; Kar, T. *J. Am. Chem. Soc.* **1995**, *117*, 6970.
14) Dunn, M. F.; Hur, O.; Leja, C. *Biochemistry* **1996**, *35*, 7378.
15) Frey, P. A.; Whitt, S. A.; Tobin, J. B. *Science* **1994**, *264*, 1927.
16) Davis, L.; Tong, H. *Biochemistry* **1995**, *34*, 3362.
17) a) Metzler, D. E.; Metzler, C. M.; Scott, R. D.; Mollova, E. T.; Kagamiyama, H.; Yano, T.; Kuramitsu, S.; Hayashi, H.; Hirotsu, K.; Miyahara, I. *J. Biol. Chem.* **1994**, *269*, 28027. b) Metzler, D. E.; Metzler, C. M.; Scott, R. D.; Mollova, E. T.; Tanase, S.; Kogo, K.; Higaki, T.; Morino, Y. *J. Biol. Chem.* **1994**, *269*, 28017 c) Kintanar, A.; Metzler, C. M.; Metzler, D. E.; Scott, R. D. *J. Biol. Chem.* **1991**, *266*, 17222. d) Metzler, C. M.; Viswanath, R.; Metzler, D. E. *J. Biol. Chem.* **1991**, *266*, 9374. e) Metzler, C. M.; Metzler, D. E.; Kintanar, A.; Scott, R. D.; Marceau, M. *Biochem. Biophys. Res. Comm.* **1991**, *178*, 385.
18) a) Austin, J. C.; Zhao, Q.; Jordan, T.; Talalay, P.; Mildvan, A. S.; Spiro, T. G. *Biochemistry*, **1995**, *34*, 4441. b) Zhao, Q.; Mildvan, A. S.; Talalay, P. *Biochemistry*, **1995**, *34*, 426. c) Holman, C. M.; Benisek, W. F. *Biochemistry* **1994**, *33*, 2672. d) Brooks, B.; Benisek, W. F. *Biochemistry* **1994**, *33*, 2682. e) Li, Y. K.; Kuliopulos, A.; Mildvan, A. S.; Talalay, P. *Biochemistry* **1993**, *32*, 1816.
19) Zhao, Q.; Abeygunawardana, C.; Talalay, P.; Mildvan, A. S. *Proc. Natl. Acad. Sci. USA.* **1996** *93*, 8220.
20) Warshel, A.; Papazyan, A.; Kollman, P. A. *Science* **1995**, *269*, 102.
21) Gerlt, J. A. *Curr. Opin. Struct. Biol.* **1994**, *4*, 593.
22) Herschlag, D.; Loh, S.; Shan, S. *Science* **1996**, *272*, 97.
23) Kato, Y.; Toledo, L. M.; Rebek, J. *J. Am. Chem. Soc.* **1996**, *118*, 8575.
24) Frey, P. A. *Science* **1995**, *269*, 104.
25) Herschlag, D. *J. Am Chem. Soc.* **1994**, *116*, 11631.
26) Cleland, W. W.; Kreevoy, M. M. *Science* **1995**, *269*, 104.
27) Shan, S.; Herschlag, D. *J. Am. Chem. Soc.* **1996**, *118*, 5515.

CARBON-CARBON BOND FORMATIONS MEDIATED BY SAMARIUM DIIODIDE

Reported by Ryan B. Prince November 14, 1996

INTRODUCTION

In the last twenty years samarium diiodide (SmI_2) has emerged as a useful reagent for reactions requiring one or two electron reductions of halides or carbonyl groups. Samarium diiodide was first applied to organic reactions by Kagan *et al.* in 1977, who established that two equiv of SmI_2 and six equiv of methyl iodide, can transform 2-octanone and methyl heptanoate into 2-methyl-2-octanol, with yields of 95 and 10 percent respectively.[1] Although the analogous Grignard reactions proceed with higher yields, these initial results sparked interest in the possible applications of this reagent. It has since been shown that SmI_2 can be used in the reduction of aldehydes, ketones[2], conjugated double bonds[2] and halides.[3] Samarium diiodide has also been used for bond cleavage reactions,[4] aldol type reactions,[5] olefin synthesis[6] and samarium Simmons-Smith cyclopropanations.[7]

Other samarium (II) salts have been used for these reactions. Samarium(II) triflate provides better diastereoselectivity in samarium-Grignard reactions and could serve as an alternative to SmI_2 for selective organic synthesis.[8a] The synthesis of $Sm(C_5H_5)_2$,[8b] $Sm(C_5Me_5)_2$,[8b] $Sm(indenyl)_2$[8c] and $Sm(fluorenyl)_2$[8c] have also been reported. These reagents offer increased solubility in organic solvents, but they have not been applied to organic reactions.

This review will cover the recent inter- and intramolecular, Reformatsky, Barbier and pinacol, carbon-carbon bond forming reactions mediated by SmI_2 and the applications of the reagent to the synthesis of natural products.

PREPARATION OF SAMARIUM DIIODIDE

Two methods are commonly used to prepare SmI_2, in an organic solvent. The first is the reaction of samarium metal with 1,2-diiodoethane to produce a mole of SmI_2 and ethylene.[1] An alternative method is the reaction of samarium metal with elemental iodine.[9] Both preparations are carried out under oxygen-free conditions, typically in tetrahydrofuran. Following the preparation

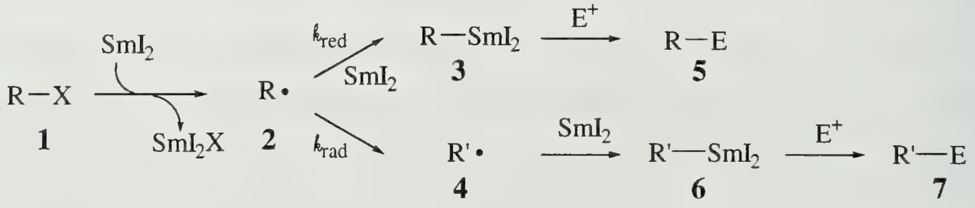
Copyright © 1996 by Ryan B. Prince

of SmI_2, the solution can either be stored under vacuum or added to the mixture containing the reaction substrates. However, tetrahydrofuran can readily donate a hydrogen atom to radicals and can ring-open to form various side products.[10] Preparations have also been reported in tetrahydropyran. The tetrahydropyran solutions of SmI_2 provide yields comparable to the tetrahydrofuran solutions, in their subsequent reactions.[11]

MECHANISM

Samarium diiodide has a large reduction potential (-1.55 V) consistent with its behavior as an effective one electron donor. The reduction of R-X is shown as the first step of Scheme 1. The high reduction potential allows for further reduction of the radical **2** to the anion **3**. In order for radical chemistry of **2** to occur, the rate of its reaction k_{rad} to give **4** must be considerably faster then the reduction of **2** to the anion **3**. The samarium organometallics from either pathway **3** or **6** can be trapped by an electrophile, to give products **5** or **7** respectively. The ratio of **5** and **7** is determined by the relative rates of k_{rad} and k_{red}. By using 6-iodo-1-hexene as the radical source **1** and para-anisaldehyde as the electrophile, Curran *et al.* were able to measure the individual rate constants k_{red} and k_{rad} for the two competing reactions of 1° radicals with SmI_2 in the presence of five equiv of HMPA.[12]

Scheme 1

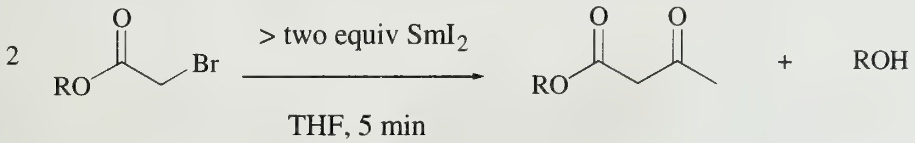


SYNTHETIC UTILITY

Samarium diiodide has been utilized as an alternative for a variety of carbon-carbon bond forming reactions, which require one or two electron reductions. The reactions often result in higher reaction yields and have a greater functional group tolerance than other commonly used one or two electron reducing agents.

Reformatsky Type Reactions

Traditional Reformatsky reactions can be used to reduce α-bromo esters to a β-ketoester. The reactions typically require the use of activated zinc and high reaction temperatures; conditions which often result in the formation of the corresponding α,β-unsaturated ester or self-condensation of the carbonyl substrate.[13] With SmI_2 it is possible to carry out Reformatsky reactions at lower temperatures and avoid the formation of unsaturated products.[14] The first use of SmI_2 in intermolecular Reformatsky type reactions was reported in 1995 and is depicted for the conversion

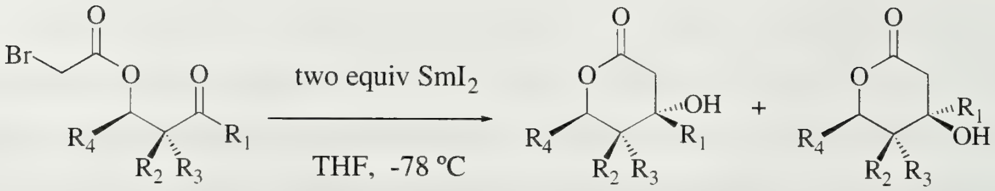


8 **9** **10**

9a R = allyl, -78 °C (70%) **9b** R = cyclohexyl, -35 °C (72%)

of **8** to **9**.[14b] It was shown that it is possible to transform several different α-bromoesters into their corresponding β-ketoesters, by using greater than two equiv of SmI_2. These reactions are complete in a few minutes and provide yields comparable to the zinc catalyzed Reformaktsy reactions, with shorter reaction times and lower temperatures. For example, α-bromoallyl acetate requires a reaction time of 18 h and results in a 86 percent yield under traditional zinc-promoted Reformatsky conditions, while the SmI_2 mediated process shown above, allows for the reaction to be completed in five minutes in 70 percent yield.[15]

Alpha-bromoesters can also be utilized in intramolecular Reformatsky type reactions.[14a,d] Molander *et al.* showed that β-bromoacetoxy ketones and aldehydes can be cyclized into β-hydroxy-δ-valerolactones with high diastereoselectivities and good yields, upon treatment with two equiv of SmI_2, as shown for the conversion of **11** to **12** and **13** in Table I. The presence of a large substituent results in a diastereoselectivity of greater than 200:1. The ratio of **12:13** can be reversed by the size and location of the substituents, as shown in the last entry of Table I. The reversal of diastereoselectivity arises from interactions between the *t*-butyl and methyl group, in the transition state leading to **12**. This samarium-mediated process gives higher yields and better diastereoselectivity than the zinc mediated, intramolecular Reformatsky reactions.[16] For example, the second entry resulted in only a 44 percent yield of the desired product, under zinc-promoted



11 **12** **13**

Table I. SmI_2 Mediated Intramolecular Reformatsky Reactions of 11

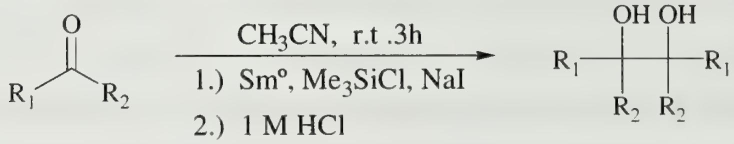
R_1	R_2	R_3	R_4	Yield (%)	Diastereomeric ratio 12:13
t-Bu	H	Me	H	85	>200:1
Me	H	H	Ph	95	>200:1
t-Bu	Me	H	Ph	88	1:>200

Reformatsky conditions.[17]

Pinacol Couplings

Traditional pinacol reactions are used to couple aldehydes or ketones to form 1,2-diols or acyloin products. They are typically performed using amalgams of sodium,[18] magnesium,[18] or aluminum[18] and suffer from slow rates, formation of alkenes or incompatibility with functional groups. Tributyltin hydride has been shown to provide moderate yields of pinacol products, in the intramolecular coupling of dialdehydes and ketoaldehydes.[19] Pinacol couplings have also been reported using a $[V_2Cl_3(THF)_6]_2[Zn_2Cl_6]$ complex.[19] This procedure allows for intermolecular, pinacol coupling of two aldehydes, but will not work for dialkyl ketones.

By using SmI_2, it is possible to couple both aliphatic and aromatic carbonyl compounds. The reagent is useful with many functional groups including amines, nitriles and nitro groups, which are not compatible with traditional pinacol coupling conditions.[21a,b] In 1993 Ishi *et al.*

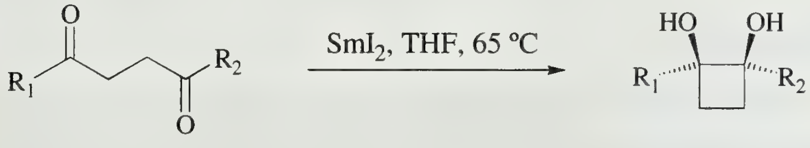


14 **15**

15a R_1 = methyl, R_2 = Ph (84%) **15b** R_1 = methyl, R_2 = n-C_4H_9 (54%)
15c R_1 = H, R_2 = Ph (89%) **15d** R = cyclopentanone (89%)

reported a procedure for coupling aliphatic and aromatic ketones and aldehydes by an *in situ* generation of SmI_2 as shown for the conversion of **14** to **15**. The product **15** was formed in a 1:1 mixture of the erythro and threo isomers.[22] By using SmI_2, couplings of both cyclic and acyclic ketones as well as aldehydes proceed in high yields, without the formation of undesired sideproducts.

Intramolecular pinacol reactions allow for the formation of well-defined carbocycles[23] and building blocks for a wide variety of molecular scaffolds.[24] By using 1,4-diketones, it is possible to synthesize *cis*-cyclobutane-1,2-diols, as shown for the conversion of **16** to **17**.[24] If the 1,4-diketone contains a cyclic moiety, the resulting product is a bicyclic cyclobutane-1,2-diol.[24] The last entry **17e** shows that a chlorine substituent survives these conditions, in contrast to the results obtained under sodium-mediated pinacol couplings, which results in the replacement of the chloride by a hydrogen.[18]

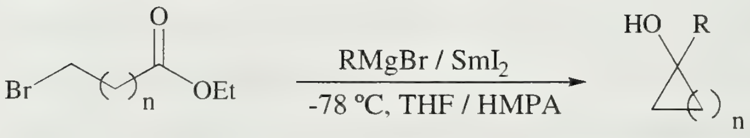


16 **17**

17a R_1 = cyclohexyl, R_2 = methyl (78%) **17b** R_1 = *i*-butyl, R_2 = methyl (81%)
17c R_1 = phenyl, R_2 = methyl (88%) **17d** R_1 = *p*-anisyl, R_2 = methyl (92%)
17e R_1 = *p*-chlorophenyl, R_2 = methyl (91%)

Barbier Reaction

Samarium diiodide Barbier reactions are analogous to the Grignard Barbier reaction. However, the SmI_2 reactions have better functional group tolerance and succeed in cases where organomagnesium or organolithium reagents fail.[2] Intermolecular reactions are limited to ketone carbonyl substrates, but are generally high yielding.[2,25a,b] By performing intramolecular samarium Barbier reactions, it is possible to synthesize 1-substituted cylopropanols and cycloalkanols,[26] as shown for **19a-d**. This reaction allows for a simple one step synthesis of 1-substituted cyclopropanols, in high yield. Traditionally these compounds have been made by a three step procedure.[27] The are several examples in the literature where samarium Barbier reactions have been used to obtain heterocyclic,[28a] bicyclic,[28b] and tricylic[28c] ring systems, in high yields. The



18 **19**

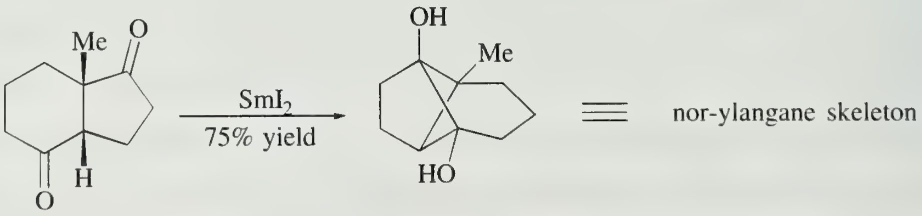
19a n = 1, R = n-C_4H_9 (99%) **19b** n = 1, R = $(CH_3)_2CH$ (85%)
19c n = 1, R = C_6H_5 (99%) **19d** n = 3, R = n-C_4H_9 (46%)

tricyclic ring system of hirsutene was obtained in 4 steps from commercially available compounds,[29] with a 15% yield compared to a 13 step total synthesis, which resulted in a 5% yield.[30]

Natural Products Synthesis

Samarium diiodide reactions have been employed as the key step in the total synthesis of several natural products.[31] The reagent has been used during the synthesis of Taxol,[31a,c] Forsolkin[31d] and (-)-C_{10}-Desmethyl Arteannuin B.[31b] The reagent provides better yields and greater selectivity and allows the formation of previously unattainable intermediates. For example, in the synthesis of (-)-C_{10}-Desmethyl Arteannuin B, using SmI_2 in the key, ring closing step of the reaction resulted in a 95% yield of the desired product with the correct stereochemistry. A previous attempt at this same reaction using a zinc-copper mediated, coupling procedure resulted in the formation of a mixture of stereoisomers, of which none of the stereoisomers could be converted to the desired product.[32]

Samarium diiodide has also been used to create the framework for monoterpenes and sesquiterpenes, as shown in the conversion of **20** to **21**.[33] The high yielding, intramolecular pinacol coupling reaction allowed for the straightforward synthesis of a highly functionalized nor-ylangane skeleton, which is a precursor to several natural products.



20 **21**

CONCLUSION

Samarium diiodide has been shown to be effective in mediating several carbon-carbon bond forming reactions. It provides an appealing alternative to classical methods as it shows improved functional group tolerance and allows reactions to occur under mild conditions. The applications of the reagent are broad and it provides easy access to cyclic, bicyclic, and tricyclic molecules and heterocycles. Recently this reagent has been employed in natural products synthesis. The use of SmI_2 is currently limited to substrates which are soluble in etheral solvents. Future work should focus on developing new conditions for the preparation of SmI_2 in organic solvents which do not result in the formation of side products. This could allow for better control of the desired products and the ability to apply the reactions to a wider variety of substrates.

REFERENCES

1. Namy, J. L.; Girard, P.; Kagan, H. B. *New J. of Chem.* **1977**, 5-7.
2. (a) Gallagher, D. J. *Organic Seminar Abstracts*, University of Illinois, June 1988, 62-71. (b) Girard, P.; Namy, J. L.; Kagan, H. B. *J. Am. Chem. Soc.* **1980**, *102*, 2693-2698.
3. Inanaga, J.; Ishikawa, M.; Yamaguchi, M. *Chem. Lett.* **1987**, 1485-1486.
4. (a) Honda, T.; Yamane, S.; Naito, K.; Suzuki, Y. *Heterocycles* **1994**, *37*, 515-521. (b) Jia, X. S.; Zhang, Y. M.; Zhou, X. J. *Tetrahedron Lett.* **1994**, *35*, 8833-8834. (c) Jia, X. S.; Zhang, Y. M.; Zhou, X. J. *Synth. Commun.* **1994**, *24*, 387-392. (d) Jia, X S.; Zhang, Y. M.; Zhou, X. J. *Synth. Commun.* **1994**, *24*, 2893-2898. (e) Chumoyer, Y.; Danishefsky, S. J.; Schulte, G. K. *J. Am. Chem. Soc.* **1994**, *116*, 11213-11228. (f) Hasegawa, E.; Takahashi, M.; Horaguchi, T. *Tetrahedron Lett.* **1995**, *36*, 5215-5218.
5. Molander, G.; Hahn, G. *J. Am. Chem. Soc.* **1986**, *51*, 2596-2599.
6. (a) Keck, G. E.; Savin, K. A.; Weglarz, M. A. *J. Org. Chem.* **1995**, *60*, 3194-3204. (b)Marko, I. E.; Murphy, F.; Dolan, S. *Tetrahedron Lett.* **1996**, *37*, 2089-2092. (c) Hart, D. J.; Wu, W. L. *Tetrahedron Lett.* **1996**, *37*, 5283-5286.
7. (a) Molander, G.; Etter, J. *J. Org. Chem.* **1987**, *52*, 3942-3944. (b) Molander, G.; Harring, L. *J. Org. Chem.* **1989**, *54*, 3525-3532.
8. (a) Fukuzawa, S.; Mutoh, K.; Tsuchimoto, T.; Hiyama, T. *J. Org. Chem.* **1996**, *61*, 5400-5405 (b) Evans, W.; Grate, J.; Choi, H.; Bloom, I.; Hunter, W. ; Atwood, J. *J. Am. Chem. Soc.* **1985**, *107*, 941-946 (c) Evans, W.; Gummersheimer, T.; Boyle, T.; Ziller, J. *Organometallics* **1994**, *13*, 1281-1284.
9. Imamoto, T.; Ono, M. *Chem. Lett.* **1987**, 501-502.
10. Souppe, J.; Namy, J.-L.; Kagan, H. B. *Tetrahedron Lett.* **1984**, *25*, 2869.
11. Namy, J. L.; Colomb, M.; Kagan, H. B. *Tetrahedron Lett.* **1994**, *35*, 1723-1726.
12. Hasegawa, E.; Curran, D. P. *Tetrahedron Lett.* **1993**, *34*, 1717-1720.
13. Ruggeri, R. B.; Heathcock, C. H. *J. Org. Chem.* **1987**, *52*, 5745.
14. (a) Molander, G. A.; Etter, J. B.; Harring, L. S.; Thorel, P.-J. *J. Am. Chem. Soc.* **1991**, *113*, 8036-8045. (b) Park, H. S.; Lee, I. S.; Kim, Y. H. *Tetrahedron Lett.* **1995**, *36*, 1673-1674. (c) Yoshida, M.; Suzuki, D.; Iyoda, M. *Synth. Commun.*

1996, *26*, 2523-2529. (d) Molander, G. A.; Etter, J. B. *J. Am. Chem. Soc.* **1987**, 6556-8. (e) Molander, G. A.; Harring, L. S. *J. Org. Chem.* **1990**, 6171-6.

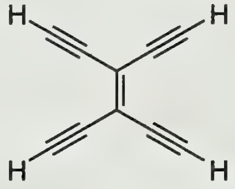
15. Kashimi, C.; Huang, X.; Harada, Y. Hosomi, A. *J. Org. Chem.* **1993**, 793-794.
16. Hannick, S. M.; Kishi, Y. *J. Org. Chem.* **1983**, *48*, 3833-3835.
17. Ukaji, Y., Kanda, H. Yamamoto, K., Fujisawa, T., *Chem. Lett.* **1990**, 597-600.
18. Kropf, H.; Thiem, J. Houben-Weyl, Methoden der Organischen Chemie, 1973; Vol. 6/1b/2.
19. (a) Hays, D., Fu, G. *J. Am. Chem. Soc.* **1995**, *117*, 7283-7284. (b) Freudenberger, J.; Konradi, A.; Pedersen, S. *J. Am. Chem. Soc.* **1989**, *111*, 8014-8016. (c) Takahara, P.; Freudenberger, J.; Konradi, A.; Pedersen, S. *Tetrahedron Lett.* **1989, *51*,** 7177-7180.
20. Furstner, A.; Csuk, R.; Rohrer, C.; Weidmann, H. *J. Chem. Soc. Perkin Trans I* **1988**, 1729-1734.
21. (a) Namy, J. L.; Souppe, J.; Kagan, H. B. *Tetrahedron Lett.* **1983**, *24*, 765-766. (b) Souppe, J.; Danon, L.; Namy, J. L.; Kagan, H. B. *J. of Organometallic Chem.* **1983**, *250*, 227-236.
22. Akane, N.; Kanagawa, Y.; Nishiyama, Y.; Ishi, Y. *Chem. Lett.* **1992**, 2431-2434.
23. Molander, G. A.; Kenny, C. *J. Org. Chem.* **1988**, 2132-4.
24. Hoffmann, H.; Munnich, I.; Nowitzki, O.; Stucke, H.; Williams, D. J. *Tetrahedron* **1996**, *52*, 11783-11798.
25. (a) Sasaki, M.; Collin, J.; Kagan, H. B. *New J. of Chem.* **1992**, *16*, 89. (b) Souppe, J.; Namy, J. L.; Kagan, H. B. *Tetrahedron Lett.* **1982**, *23*, 3497.
26. Fukuzawa, S.; Furuya, H.; Tsuchimoto, T. *Tetrahedron* **1996**, *52*, 1953-1960.
27. Barluenga, J.; Florez, J.; Yus, M. *Synthesis* **1983**, 643.
28. (a) Ha, D. C.; Yun, C. S.; Yu, E. *Tetrahedron Lett.* **1996**, *37*, 2577-2580. (b) Molander, G. A.; Etter, J. B.; Zinke, P. W. *J. Am. Chem. Soc.* **1987**, 453-63. (c) Molander, G. A.; Harris, C. R. *J. Am. Chem. Soc.* **1996**, *118*, 4059-4071.
29. Molander, G. A.; Harris, C. R. *J. Am. Chem. Soc.* **1996**, *118*, 4059-4071
30. Curran, D.; Chen, M. *Tetrahedron Lett.* **1985**, *26*, 4991.
31. (a) Swindell, C. S.; Chander, M. C.; Heerding, J. M.; Klimko, P. G.; Rahman, L. T.; Raman, J. V.; Venkataraman, H. *Tetrahedron Lett.* **1993**, *34*, 7005-7008. (b) Schwaebe, M.; Little, R. D. *J. Org. Chem.* **1996**, 3240-4. (c) Swindell, C. S.; Fan, M. *Tetrahedron Lett.* **1996**, *37*, 2321-2324. (d) Anies, C.; Pancrazi, A.; Lallemand, J. Y. *Tetrahedron Lett.* **1994**, *35*, 7771- 7774.
32. Dreiding, A.; Goldberg, O.; Deja, I.; Rey, M *Helv. Chim. Acta* **1980**, 2455-2468.
33. Nowitzki, O.; Munnich, I.; Stucke, H.; Hoffmann, H. *Tetrahedron* **1996**, *52*, 11799-11810.

TETRAETHYNYLETHENE: A MOLECULAR ARCHITECT'S APPROACH TO CARBON RICH FRAMEWORKS

Reported by Gregory N. Tew November 18, 1996

INTRODUCTION

Tetraethynylethene (TEE, **1**) and its derivatives have provided access to novel and interesting cross conjugated systems and new carbon rods and scaffoldings. The two common allotropes of carbon, graphite and diamond, have remarkable properties which are valuable in many applications.[1-3] Graphite is a 2-dimensional sheet and diamond is a 3-dimensional infinite network. In addition to these natural occurring forms of carbon, several groups have predicted structures that could lead to 2- or 3-dimensional carbon networks.[4,5] The unsaturated hydrocarbon **1** may be a precursor to new infinite carbon networks.



1

Hori et al. reported the first derivatives of tetraethynylethene in 1969.[6] Six years later, Hauptmann prepared three additional derivatives of tetraethynylethene, one of which is a direct precursor to tetraethynylethene.[7] However, the first synthesis of tetraethynylethene was reported in 1991 by Diederich and co-workers.[8] Following the initial report of this synthesis, Diederich and co-workers have prepare a number of different carbon frameworks using TEE as the molecular building block. Their systems include expanded dendralenes, expanded radialenes, dehydroannulenes, oligotriacetylenes and others.[9,10] This review will focus on the synthesis of TEE and highlight the concepts of cross conjugation, acetylene stability, and applications of TEE toward interesting carbon frameworks.

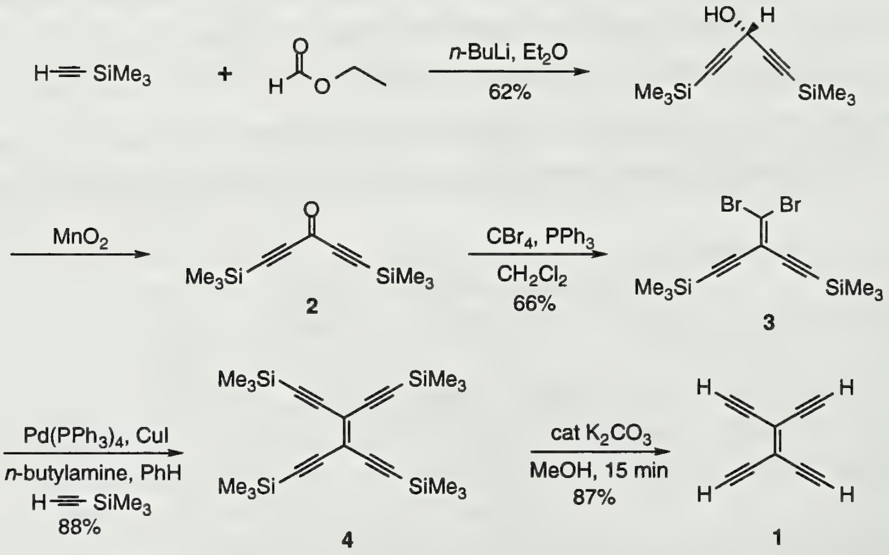
SYNTHESIS OF TETRAETHYNYLETHENE

Diederich et al. prepared 1,5-bis(trimethylsilyl)-1,4-pentadiyn-3-one (**2**) by oxidation of the corresponding alcohol (Scheme 1).[7] Using Corey-Fuchs conditions, compound **2** was converted to the dibromoolefin **3** followed by palladium catalyzed alkynylation with trimethylsilylacetylene in the presence

Copyright © 1996 by Gregory N. Tew

of catalytic CuI to afford the persilylated TEE derivative **4**. Compound **4** is stable in the crystalline form and in pentane solution at -20 °C up to several months. Deprotection of **4** with catalytic K_2CO_3 in MeOH afforded TEE **1** which crystallized out of pentane at -10 °C as white plates and rapidly polymerized to a black solid when warmed to room temperature even in the absence of O_2.[8,11]

Scheme 1

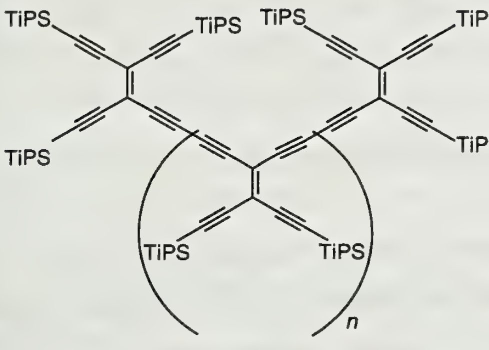


The hydrocarbon **1** was characterized by ^{1}H NMR, ^{13}C NMR, IR, UV/Vis and HR-MS. The IR is especially simple with a strong absorption at 3307 cm^{-1} (C-H stretch), a weak signal at 2102 cm^{-1} (C≡C stretch), and two strong absorptions at 653 and 632 cm^{-1} (coupled C-H bending). Diederich and co-workers have extended this methodology to the synthesis of a variety of differentially deprotected derivatives. They have prepared *trans*, *cis*, and *gem* bisdesilylated compounds to provide a number of different carbon rich frameworks.

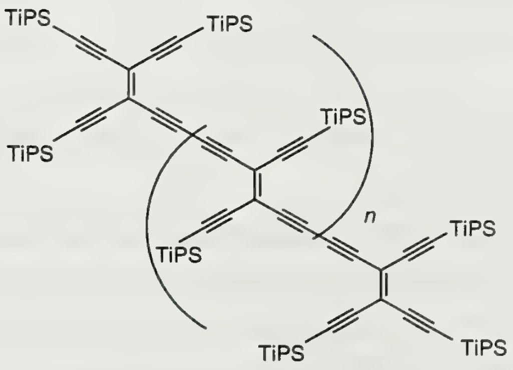
EXPANDED DENDRALENES: CROSS CONJUGATED CHROMOPHORES

The synthesis of *gem* bisdesilylated compounds led to a novel class of cross conjugated chromophores known as expanded dendralenes. Dendralenes represent a class of fully cross conjugated polyolefins. The expanded dendralenes have a diacetylene fragment inserted between each pair of double bonds in the analogous dendralene.[12] The cross conjugated chromophores **5** and **6** were prepared along with their linear isomers **8-9** and are stable to air at room temperature for several months.

Scheme 2



5 and 6



7-9

Compound	n	λ_{max} (nm)
4	~	350
5	1	444
6	2	444

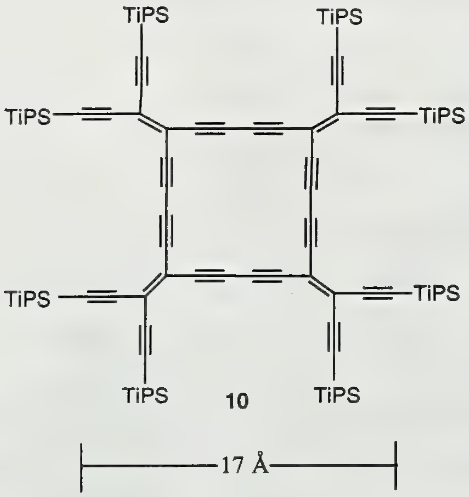
Compound	n	λ_{max} (nm)
7	0	432
8	1	479
9	2	497

Cross conjugation occurs in a compound with three unsaturated units where two units are not conjugated with each other but each is conjugated with the third.[13] These novel cross conjugated compounds and their linearly conjugated isomers provide a set of compounds for the systematic investigation of π-electron delocalization efficiency. Comparison of the electronic spectra for compounds **4** and **7-9** clearly illustrates that as the length of the linear conjugated segment increases the longest λ_{max} shifts toward the red. This indicates increased π-delocalization as the length of the chromophore increases. However, the longest λ_{max} for **5** and **6** remains constant though the number of π-electrons in the system increases. Thus, for **5** and **6** the π-delocalization is only efficient through the longest linear fragment (dodeca-3,9-diene-1,5,7,11-tetrayne). CPK and preliminary computer models for the cross conjugated systems **5** and **6** suggest that steric repulsion between triisopropylsilyl groups bend the chromophore out of plane. Diederich et al. attribute the inefficient π-delocalization to cross conjugation, not out-of-plane bending of the chromophore. In the absence of an X-ray crystal structure, higher level calculations are currently underway to further investigate the planarity of these chromophores.[12] Substantial vibronic fine structure seen in the UV/Vis spectra suggests these molecules are rigid in solution. Several related compounds exhibit significant vibrational structure in their UV/Vis spectra and the X-ray structure shows they are planar.[14,15] By analogy, it is possible to suggest these expanded dendralenes are planar and cross conjugation must limit the π-delocalization. Diederich et al. suggest the stability of these compounds arises from the inefficient cross conjugation. This cross conjugated stability may represent a general stabilizing principle of unsaturated carbon systems like graphite and buckminsterfullerene.[12]

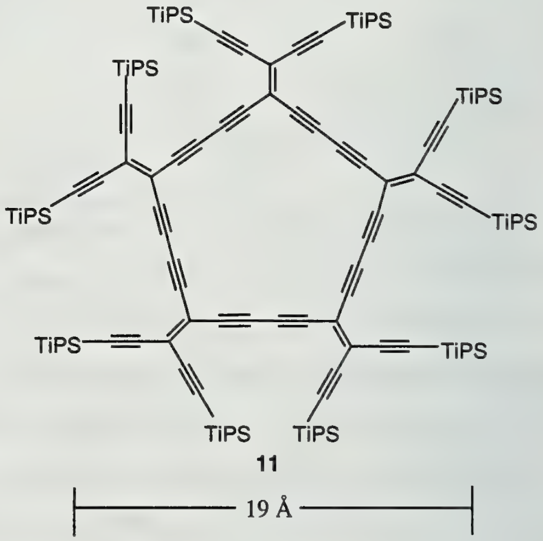
EXPANDED RADIALENES: CROSS CONJUGATED MACROCYCLES

A set of cyclic chromophores, expanded radialenes, were prepared from linearly extended TEE derivatives (Scheme 3). Radialenes are a class of all-*exo*-methylene cycloalkanes of molecular formula C_nH_n. Expanded radialenes are formed when diacetylene units are inserted between each pair of vicinal *exo*-methylenes in the cyclic framework and have a molecular formula of $C_{3n}H_n$.[16] The macrocycles **10**-**12** are possible precursors to infinite all carbon 2- dimensional sheets.

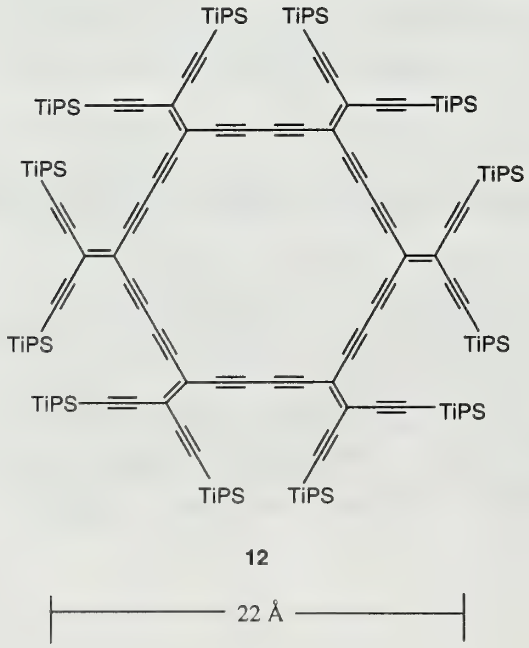
SCHEME 3



10



11



12

Compound	λ_{max} (nm)
10	451
11	441
12	446

All three macrocycles are yellow solids that are soluble in most aprotic solvents. These compounds are stable with melting points above 220 °C. The dimensions of the carbon macrocycles are 17 Å (**10**), 19 Å (**11**), 22 Å (**12**), not including the TiPS groups. The appearance of vibrational structure in the electronic absorption spectra suggests the molecules are rigid and efficient π-delocalization is limited to the longest linear segment. A comparison of the longest λ_{max} between **7** and the three macrocycles suggests dodeca-3,9-diene-1,5,7,11-tetrayne is the longest linear segment. The three macrocycles show little shift in their longest λ_{max} and AM1 calculations indicate they are distorted significantly from planarity.[15] Diederich et al. suggest inefficient π-delocalization due to cross conjugation to explain the electronic data.

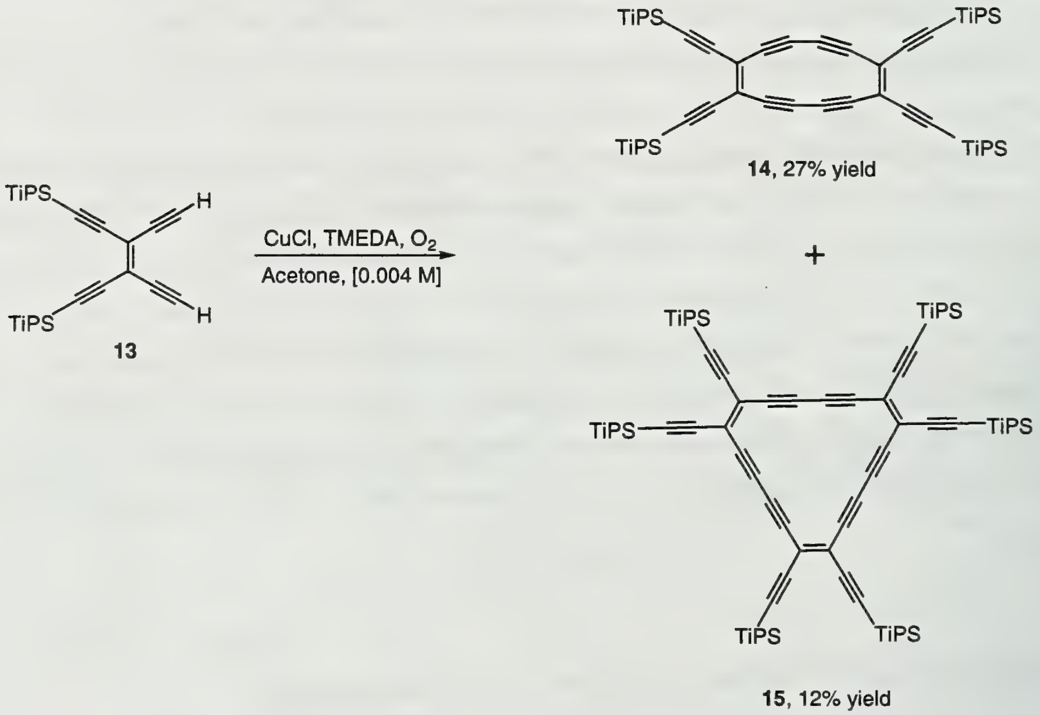
Another explanation of the small change seen in λ_{max} is that the macrocycles are bent out of plane limiting the conjugation to the longest linear segment (dodeca-3,9-diene-1,5,7,11-tetrayne). A small delocalization is associated with the cyclic structures with **10** having the longest λ_{max} at 451 nm of the three macrocycles. Compound **10** is suggested to be the most planar of the three structures and cyclic voltammetry supports a more π-delocalized system.[15] The macrocycles could show vibrational structure in the UV/Vis specta if they are not planar but confined to a rigid nonplanar conformation. Until the conformation of the expanded radialenes can be elucidated, the explanation of the electronic spectra cannot exclude cross conjugation or nonplanarity of the macrocycles as the mechanism of inefficient π-delocalization.

DEHYDROANNULENES: AROMATIC AND ANTIAROMATIC

Two novel dehydroannulenes **14** and **15** were prepared from oxidative coupling of the TEE *cis*-bissilylprotected *cis*-bisdeprotected **13**.[17] Annulenes and dehydroannulenes have received a considerable amount of attention from physical organic chemists as tests of the concepts of aromaticity.[18] Crystals of **14** are stable when exposed to air at room temperature, they are deep-purple in color, and melt at 200 °C. However, if **14** is concentrated without crystallization significant decomposition occurs. Compound **15** is a bright yellow stable solid that melts at 215 °C. The planarity of **14** and **15** is clearly demonstrated by the X-ray crystal structures. These two planar cyclic molecules upon desilylation could serve as precursors to infinite 2-dimensional carbon networks.

Based on Hückel theory, the planar, cyclic tetradehydro[12]annulene **14** is predicted to be antiaromatic while the planar, cyclic hexadehydro[18]annulene **15** is aromatic. A careful investigation of the UV/Vis spectra and comparison to related known dehydroannulenes provisionally assigned antiaromaticity to **14** and aromaticity to **15**. The electronic spectrum of **14** showed a weak absorption near 660 nm (1.87 eV) supporting the existence of a low lying antiaromatic HOMO-LUMO gap.[19] The longest absorption for **15** appeared near 480 nm (2.57 eV) and is consistent with a higher energy HOMO-LUMO gap for an aromatic compound. The electronic spectra of **14** and **15** closely resemble those of previously characterized dehydroannulenes which are assigned aromatic or antiaromatic character. The UV/Vis study presented here suggests that careful examination of the electronic spectra and comparison with other known compounds may be a satisfactory method for assigning the aromatic character of ring systems when proximal hydrogens are absent.

SCHEME 4



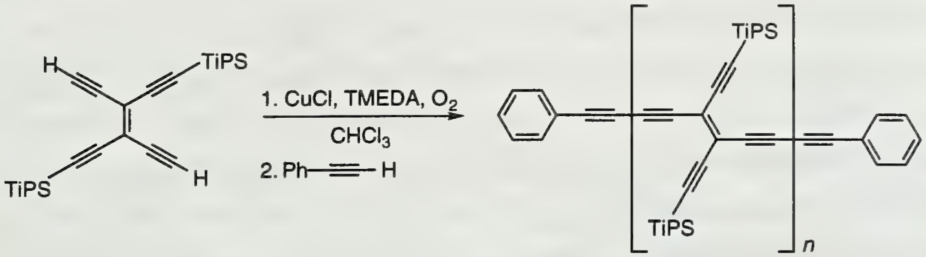
The proton shifts in ^{1}H NMR investigations of derivatives of **14** and **15** provided further evidence supporting the aromatic/antiaromatic assignments.[15] These derivatives have *tert*-butyldimethyl silyloxymethylene groups replacing every *exo*-acetylene unit. The methylene protons in the acyclic precursor resonant at 4.34 ppm. The methylene protons in the tetradehydro[12]annulene derivative resonant at 3.61 ppm while in the hexadehydro[18]annulene they are at 4.83 ppm.

CONJUGATED CARBON RODS: THE POLYTRIACETYLENE BACKBONE

Preparation of nanometer sized molecular rods for use in molecular scaffolding and molecular devices has increased rapidly in the last decade.[20] However, a major problem with the study of many rigid conjugated oligomers and polymers is their insolubility. The rods shown in Scheme 5 possess the novel polytriacetylene (PTA) backbone, they are stable to oxidation and readily soluble in common organic solvents so that their properties can be thoroughly investigated.[14]

The electronic spectra of the oligomers **16-20** show a red shift with increasing length and significant vibrational structure consistent with a rigid backbone. In addition, a plot of the longest absorption λ_{max} versus the inverse of the oligomer length allowed the band gap of the infinite polymer to be predicted at 540 nm (2.29 eV).[12] The infinite polymer is defined as the length the polymer must be so the end groups do not effect the electronic or optical properties.[21] An experimental value of 591 nm (2.1 eV) for a polymer of M_n = 9600 and an average degree of polymerization = 22 was determined. This demonstrates the benefit of studying a small oligomer to gain understanding of the a polymer. However, caution must be used in such predictions because not all properties scale linearly with increasing length. For example, the first reduction potential for the PTA series does not scale linearly. A plot of the reduction potentials for the five oligomers **16-20**, indicates the reduction for the infinite polymer should occur at -1.01V but was experimentally determined to be -0.70V.[21]

SCHEME 5



Compound	**16**	**17**	**18**	**19**	**20**
n	1	2	3	4	5
length (Å)	19.4	26.8	34.3	41.8	49.2
% yield	28	33	16	7	2

CONCLUSIONS

Following the initial report of tetraethynylethene, Diederich and co-workers have prepared a variety of novel structural architectures using TEE as a building block. They have demonstrated that stable, highly soluble nanometer sized carbon rich molecules can be synthesized in the laboratory. A systematic study of novel chromophores has suggested cross conjugation as an inefficient pathway for π-delocalization and results in increased compound stability. Recent work suggests interesting electronic and optical properties can be generated from TEE carbon backbones including second and third NLO signals.[22-24] Development of these discrete organic molecules into new infinite carbon networks is a future goal for this research. The developments in this field and the technological importance of graphite and diamond encourage the pursuit of new carbon allotropes.

REFERENCES

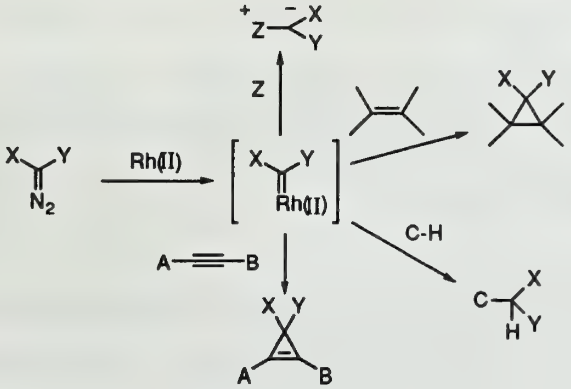
1) Field, J. E. *The Properties of Diamond*; Academic Press: New York, 1979.
2) Paxton, R. R. *Manufactured Carbon: A Self-Lubricating Material For Mechanical Devices*; CRC Press, Inc: Boca Raton, 1979.
3) C_{60} has been discovered in nature. The current source of C_{60} is synthetic production by resistive or arc heating of graphite.
4) Diederich, F.; Rubin, Y. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1101-1123.
5) Hoffmann, R.; Hughbanks, R.; Kertesz, M.; Bird, P. H. *J. Am. Chem. Soc.* **1983**, *105*, 4831-4832.
6) Hori, Y.; Noda, N.; Kobayashi, S.; Taniguchi, H. *Tetrahedron Lett.* **1969**, 3563.
7) Hauptmann, H. *Tetrahedron* **1976**, *32*, 1293.
8) Rubin, Y.; Knobler, C. B.; Diederich, F. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 698-700.
9) Diederich, F. *Nature* **1994**, *369*, 199-207.
10) Stang, P. J.; Diederich, F. *Modern Acetylene Chemistry*; VCH: New York, 1995.
11) Anthony, J.; Boldi, A. M.; Rubin, Y.; Hobi, M.; Gramlich, V.; Knobler, C. B.; Seiler, P.; Diederich, F. *Helv. Chim. Acta* **1995**, *78*, 13-45.
12) Boldi, A. M.; Anthony, J.; Gramlich, V.; Knobler, C. B.; Boudon, C.; Gisselbrecht, J.-P.; Gross, M.; Diederich, F. *Helv. Chim. Acta* **1995**, *78*, 779-796.
13) March, J. *Advanced Organic Chemistry*; 4th ed.; Wiley: New York, 1992.
14) Anthony, J.; Boudon, C.; Diederich, F.; Gisselbrecht, J.-P.; Gramlich, V.; Gross, M.; Hobi, M.; Seiler, P. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 763-766.
15) Boldi, A. M.; Anthony, J.; Boudon, C.; Gisselbrecht, J.-P.; Gross, M.; Seiler, P.; Knobler, C. B.; Diederich, F. *Helv. Chim. Acta* **1995**, *78*, 797-817.
16) Boldi, A. M.; Diederich, F. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 468-471.
17) Anthony, J.; Knobler, C. B.; Diederich, F. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 406-409.
18) Sondheimer, F. *Acc. Chem. Res.* **1972**, *5*, 81-91.
19) Minkin, V. I.; Glukhovtsev, M. N.; Simkin, B. Y. *Aromaticity and Antiaromaticity: Electronic and Structural Aspects*; Wiley: New York, 1994.
20) Tour, J. M. *Chem. Rev.* **1996**, *96*, 537-553.
21) Schreiber, M.; Anthony, J.; Diederich, F.; Spahr, M.; Nesper, R.; Hubrich, M.; Bommeli, F.; Degiorgi, L.; Wachter, P.; Kaatz, P.; Bosshard, C.; Gunter, P.; Colussi, M.; Suter, U. W.; Boudon, C.; Gisselbrecht, J.-P.; Gross, M. *Adv. Mater.* **1994**, *6*, 786-790.
22) Tykwinski, R. R.; Schreiber, M.; Gramlich, V.; Seiler, P.; Diederich, F. *Adv. Mater.* **1996**, *8*, 226-231.
23) Bosshard, C.; Spreiter, R.; Gunter, P.; Tykwinski, R. R.; Schreiber, M.; Diederich, F. *Adv. Mater.* **1996**, *8*, 231-234.
24) Tykwinski, R. R.; Diederich, F. Personal Communication, 1996 .

MECHANISM OF ASYMMETRIC INDUCTION FOR CARBENOID TRANSFORMATIONS USING CHIRAL RHODIUM(II) CATALYSTS

Reported by David R. Anderson December 2, 1996

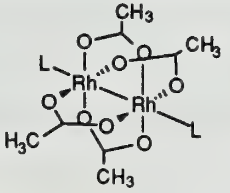
INTRODUCTION

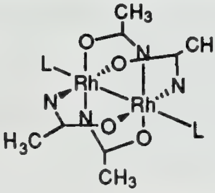
Carbenes as reactive, neutral, divalent carbon species have generally been of limited synthetic value owing to their indiscriminate reactivity. Metallocarbenes, however, have selective reactivity and have emerged as very useful synthetic intermediates in the past 20 years.[1] Metallocarbenes are typically generated by the transition metal catalyzed decomposition of α-diazocarbonyl compounds. While many transition metals have been investigated, Rh(II) salts have become the catalysts of choice for inter- and intramolecular cyclopropanations, cyclopropenations, insertions, and ylide formation.[2] The use of chiral rhodium catalysts to provide carbenoids for these transformations has the potential of converting prochiral reactants into enantioenriched chiral products.[3] This review will focus on rhodium catalysts that have recently been developed and the mechanism of asymmetric induction for these processes.



CATALYST STRUCTURE AND CATALYTIC CYCLE

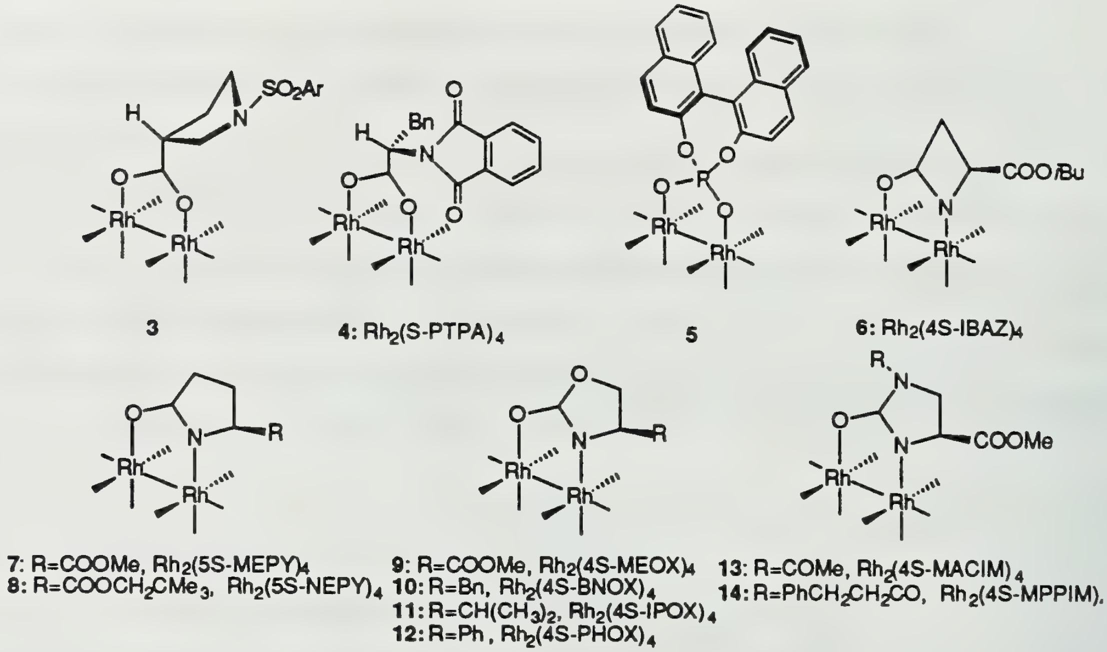
The structure of $Rh_2(OAc)_4$ (**1**), and its amide analog $Rh_2(acam)_4$ (**2**) are depicted below. Rhodium(II) is a d^7 transition metal and typically forms Rh-Rh dimers. The ligands attached to the Rh-Rh dimer are categorized into bridging ligands and axial ligands. The bridging ligands of **2** adopt a (2,2-cis) geometry.[4] The axial ligands, L, are weakly held by the rhodium and dissociate readily. The chemoselectivity of rhodium catalyzed carbenoid reactions is generally dependent on the electronic nature of the bridging ligands.[5,6] Rhodium complexes with carboxylate or carboxamide ligands are easily prepared by ligand exchange of **1** driven to completion by removal of acetic acid.


1


2

Copyright © 1996 David R. Anderson

Exchange of acetate for chiral ligands produces chiral rhodium complexes. To date, a variety of chiral carboxylic acids,[7-11] binapthyl phosphates,[12] pyrrolidinones, oxazolidinones,[4] and imidazolidinones[13] have been used with varying success in asymmetric carbenoid transformations. The structures of these catalysts along with the pertinent acronyms are shown in Figure 1 with three of the ligands and one of the enantiomers omitted for clarity.



3

4: $Rh_2(S\text{-}PTPA)_4$

5

6: $Rh_2(4S\text{-}IBAZ)_4$

7: R=COOMe, $Rh_2(5S\text{-}MEPY)_4$
8: R=$COOCH_2CMe_3$, $Rh_2(5S\text{-}NEPY)_4$

9: R=COOMe, $Rh_2(4S\text{-}MEOX)_4$
10: R=Bn, $Rh_2(4S\text{-}BNOX)_4$
11: R=$CH(CH_3)_2$, $Rh_2(4S\text{-}IPOX)_4$
12: R=Ph, $Rh_2(4S\text{-}PHOX)_4$

13: R=COMe, $Rh_2(4S\text{-}MACIM)_4$
14: R=$PhCH_2CH_2CO$, $Rh_2(4S\text{-}MPPIM)$.

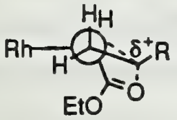
Figure 1: Catalyst structures and acronyms.

The proposed catalytic cycle of rhodium catalyzed decomposition of diazo compounds involves initial displacement of one of the axial ligands with the diazo compound generating a zwitterionic intermediate which loses nitrogen to form a metallocarbene.[2] The metallocarbene can be regarded as a stabilized carbocation; the extent of backbonding from the rhodium is governed by the electron donating or electron withdrawing effects of the ligands on the catalyst.[14] Nucleophilic attack by the olefin, alkyne, heteroatom, or sigma bond of the substrate results in the transfer of the carbenoid species and regeneration of the catalyst. Although no rhodium(II) carbene complex has been isolated or detected spectroscopically, their presence in the catalytic cycle is consistent with the products observed from these reactions.

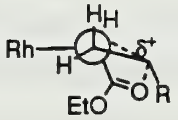
Typical reaction conditions for these transformations are mild and products are isolated by distillation or chromatographic techniques. The diazo compound is slowly added to a solution of the olefin and 0.5-1 mol % catalyst in dichloromethane via syringe pump. Slow addition is required to avoid the creation of carbene dimers, and anhydrous conditions are required to avoid the alcohols and ethers which result from water insertion of the metallocarbene. Catalyst turnovers have been estimated at 1000, but no estimate of the feasibility of catalyst reuse has been made with the exception of one chiral polyethylene bound catalyst which can be used up to seven times.[15]

INTERMOLECULAR CYCLOPROPANATION REACTIONS

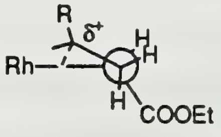
Enantioenriched cyclopropanes are synthetic targets as this ring system is present in a number of natural products.[16] These compounds are useful precursors to functionalized 7-membered rings[17] and may be used to create β-strand peptide mimics.[18] Although homogeneous chiral copper catalysts have been successfully used for intermolecular cyclopropanations for 20 years, development of chiral rhodium catalysts has only recently begun.[3,19] Chiral copper catalysts have been used to cyclopropanate olefins in high yields and good ee's, but give poor diastereoselectivity, affording mixtures of cis and trans cyclopropanes. The trans isomer is favored in most cases and this preference is exacerbated with the use of large ester groups on the diazoacetate. The majority of rhodium catalysts demonstrate an inherent selectivity for the trans isomer, but modification of the bridging ligands has been shown to overcome this preference.



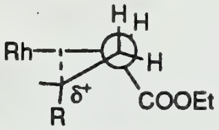
15



16

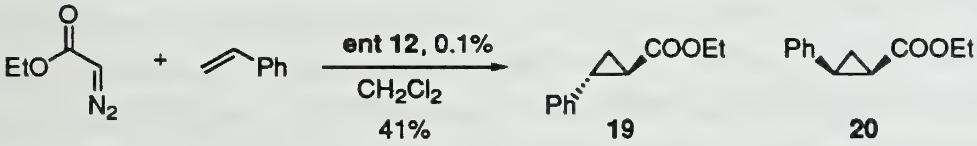


17



18

In the proposed mechanisms of cyclopropanation of an olefin by a metallocarbene, the less substituted end of the olefin approaches the carbenoid and makes a stronger partial bond than the more substituted end. The developing positive charge is stabilized in the transition state through interaction with the ester group and cyclopropane formation is completed by backside displacement of the metal.[2] The transition states leading to the trans and cis isomers are shown as **15** and **16** respectively. Transition state **15** is presumed to be lower in energy than **16** owing to interactions between the ester and the olefin substituent. Although frontside electrophilic substitution transition states **17** and **18** are predicted to be preferred by FMO theory, steric interactions with the face of the catalyst are assumed to disfavor these transition states. Moreover, cyclopropanations with stoichiometric iron carbenoid complexes have been shown to occur through backside electrophilic substitution.[20]



19 : **20**

1 : 2

24% ee 57% ee

Reaction of ethyl diazoacetate with styrene in the presence of **ent-12** gives a 1:2 mixture of **19** (24% ee) and **20** (57% ee).[21] The proposed transition states for this reaction are depicted in Figure 2. The face of **ent-12** can be divided into 4 quadrants. The phenyl groups in **ent-12** create the sides of a half-box in which the reaction takes place. The coordinated carbene is considered to have 2 low energy transition state conformations shown as **21, 22** and **23, 24**. The olefin can approach the carbenoid with either its *si* or *re* face leading to the observed products. The distribution of products is dictated by the relative energies of the transition states, and **22** is favored based on steric interactions. The preference for cis substituted cyclopropanes is rationalized by assuming that **22** is favored over **21** and **23** over **24**.

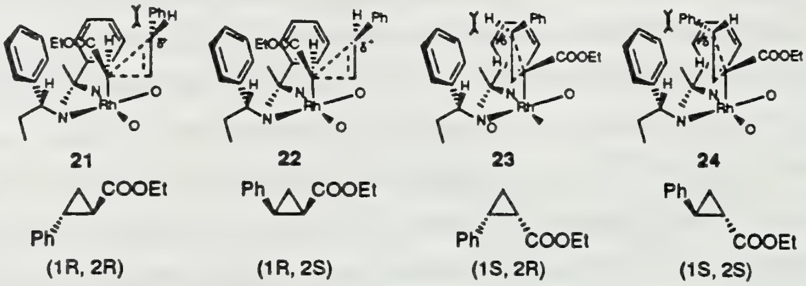
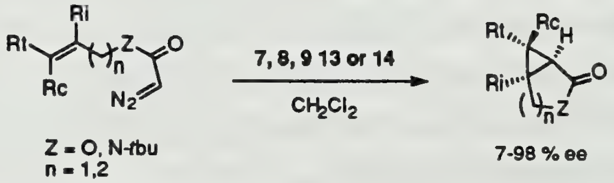


Figure 2: Proposed transition states for the reaction of ethyl diazoacetate with styrene using **ent-12.**

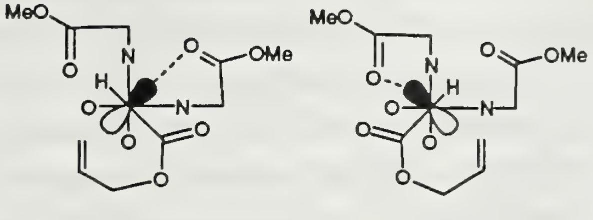
The sterically bulky groups in **12** accomplish the desired reversal in diastereoselecetivity, but they also have decreased the enantioselectivity of the catalyst and products are obtained in low yield. Newly developed catalyst **6** also displays a modest reverse in diastereoselectivity, and yields and enantioselectivities are much improved.[22]

INTRAMOLECULAR CYCLOPROPANATION REACTIONS

Chiral rhodium catalysts have been demonstrated to catalyze intramolecular cyclopropane formation of allylic and homoallylic diazoacetates and diazoacetamides in good yields and enantioselectivities.[23,24] The tether restricts the approach of the olefin and only one diastereomer is produced.



In the case of allylic diazoesters, (Z = O, n=1) the enantioselectivity of the reaction is very sensitive to the substitution pattern of the olefin. The presence of a cis substituent (Rc = methyl, ethyl, isopropyl, isobutyl, phenyl, benzyl) greatly enhances the enantioselectivity of the reaction. Products were obtained in good yields (>75 %) and in good ee's (>94 %) when **7** was used as the catalyst. When there is a trans substituent (Rt = methyl, propyl, isopropyl, phenyl) the ee's decrease with increasing size of the substituent. Substitution at Ri resulted in loss of enantiocontrol and only 7 % ee is observed with **7**. Sterically hindered catalysts **13** and **14** give better results than **7** for this substitution pattern, and the product is obtained in excellent yield (90%) and moderate ee (<78 %). Homoallylic substrates are less sensitive to changes in substitution and give slightly lower ee's attributed to greater conformational flexibility. To account for these results, Doyle and coworkers have developed the model shown in Figures 3 and 4.[24]



25 26

Figure 3: Clockwise versus counterclockwise approach of the olefin to the carbenoid with catalyst **7**.

The face of the catalyst can be considered to be divided into four quadrants with the carbenoid presumed to have two low-energy conformations, **25** and **26**. The carbenoid is stabilized by interaction with the carbonyl groups of the ligands and the olefin can be considered to approach from the side opposite to the stabilizing group. If the orientation of the olefin with respect to the face of the catalyst is the same in **25** and **26**, these approaches lead to different enantiomers. Of these two conformers, **25** is expected to lead to the favored transition state due to the fact that the olefin approaches the carbene from the most "open" quadrant of the catalyst face. Interactions between the olefin and the ligand protruding from the face of the catalyst raise the energy of transition states derived from **26**.

The orientation of the double bond in the transition state is suggested by Doyle to be dictated by the substitution pattern on the olefin and the topography of the catalyst face. The two orientations of the olefin with respect to the carbenoid with a clockwise approach of the olefin are depicted in Figure 4 as **27** and **28**. The analogous arrangements of the olefin with a counterclockwise approach are depicted as **31** and **32**. The major enantiomer observed with **7** is **29**. With large substitutents at the Rc position, this model correctly predicts that **27** would be the preferred conformation and destabilization of **28** will lead to improved enantioselection. Similarly, larger Rt substitutents will lead to steric interactions with the catalyst face and hence degrade enantioselectivity by attenuating the energy difference between **27** and **28**. Large groups at the Ri position resulted in no enantioselectivity using **7**; steric interactions with the catalyst face raise the energy of **27** and it is suggested reaction by way of **28** becomes competitive.

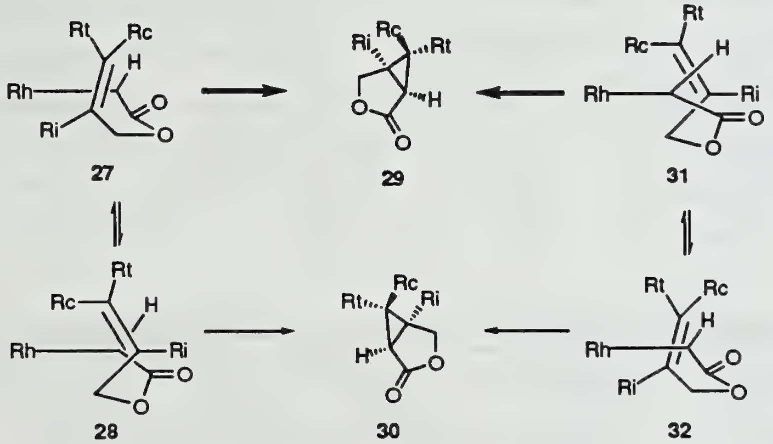
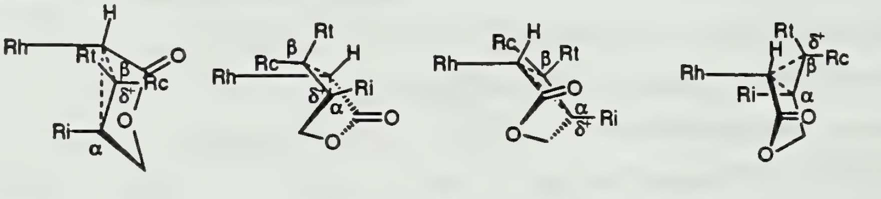


Figure 4: Doyle's proposed transition states for intramolecular cyclopropanation.

The conformational structures Doyle has proposed can be drawn into transition states. Possible transition state geometries leading from **27**, **28**, **31** and **32** are depicted in Figure 5 as **33**, **34**, **35** and **36** respectively. Conformation **27** can lead to transition state **33** which is chair-like. The α-carbon of the double bond interacts with the carbenoid and partial bond formation leaves a partial positive charge at the carbon denoted β. The Rh-C bond then is subjected to backside electrophilic attack by the electron poor β-carbon leading to the observed product with the correct stereochemistry. Reaction from conformation **28** leads to the boat - like transition state **34**. The distal end of the double bond forms a partial bond with the carbenoid leaving a partial positive charge at the α-carbon. Due to the geometry of the transition state **34**, only backside electrophilic substitution at the carbenoid carbon is available leading to the minor product **30**. Thus the observed products can be rationalized as a competition through boat and chair-like transition states and charge stabilization at the α- or β-carbon.



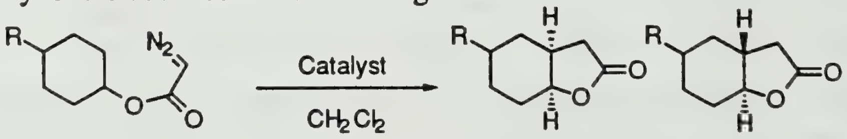
33 **34** **35** **36**

Figure 5: Possible transition states for intramolecular cyclization of allylic lactones.

INTRAMOLECULAR C-H BOND INSERTIONS

The insertion of a carbenoid into an unactivated C-H bond holds great promise as a synthetic tool, and the possibility of executing this transformation asymmetrically adds to its utility. In such transformations regioselectivity in addition to diastereoselectivity and enantioselectivity must be controlled. While the formation of 5-membered rings is usually observed,[25] conformational biases and electronic influences of neighboring heteroatoms may lead to different products.[26,27] Doyle has recently reported that the decomposition of diazoacetates in the presence of catalysts **7**, **9** and **13** and their enantiomers give products of C-H insertion with excellent regio-, diastero-, and enantioselectivity.[28,29]

The selectivity of these reactions was demonstrated by examining the products obtained from the reaction of **37** in the presence of **7**, **9**, and **13**. The sterically bulky catalyst **13** gave 5-membered lactones in excellent diastereoselectivity and enantioselectivity. Investigation of the conformationally biased 4-cis- and 4-trans cyclohexyl diazo acetates lent some insight into the mechanism of the selectivity.



37

Catalyst	R	Cis:Trans	% ee cis	% ee trans
7	H	75:25	97	91
9	H	55:45	96	95
13	H	99:1	97	65
13	cis CH_3	99:1	95	-
13	trans CH_3	63:37	93	57

The mechanism of C-H insertion is suggested to be electrophilic attack of the empty p orbital of the carbenoid on the σ C-H bond. The formation of new C-C and C-H bonds is synchronous with elimination of the catalyst. Doyle has proposed the model depicted in Figure 6 to account for the observed selectivity. As before, the ligands of **13** favor the clockwise approach of the substrate to the carbenoid. The two orientations of the cyclohexane ring with respect to the carbene are depicted as **38** and **39**. Of the two transition states leading from these conformations, the one derived from **38** should be higher in energy due to steric interactions with the bulky face of **13**. A 4-cis *t*-butyl group reinforces the preferred conformation **39**, while a 4-trans t-butyl group would raise the energy of the transition state derived from **39** and other boat like conformations may lead to competitive pathways.

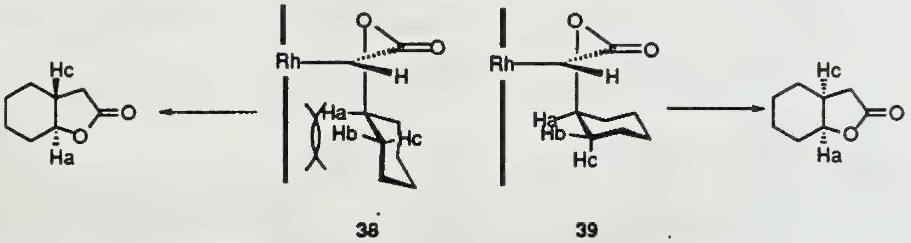
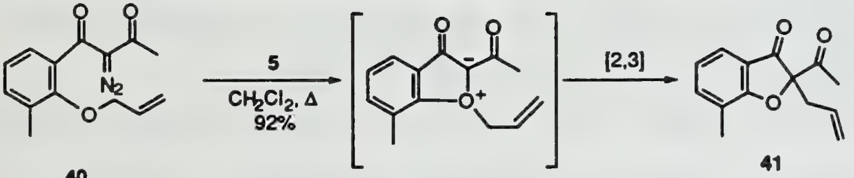


Figure 6: C-H bond insertion transition states.

While this model serves well to explain these results, it fails to account for Müller's observation that the analogous reaction using an α-diazoketone instead of the diazoacetate afforded a racemic mixture of the cis products using **7**.[30] This result implies that the ester oxygen is critical to the enantioselectivity of the reaction; the model proposed in Figure 6 does not provide a rational for this result.

YLIDE FORMATION

Carbenoids also react with heteroatoms to form ylides which exhibit a variety of useful reactivity including [2,3] sigmatropic rearrangements and dipolar addition reactions.[31] In these transformations, the catalyst is presumed to detach from the ylide prior to its conversion to product. However, the use of catalyst **5** in the decomposition of **40** gave **41** in 92% yield and 30% ee.[32] The mechanism of asymmetric induction and the role the catalyst plays in this reaction is unknown.



40

41
30% ee

CONCLUSION

Chiral dirhodium carboxamide catalysts have been demonstrated to be effective for a number of carbenoid transformations of diazoacetates and diazoacetamides including cyclopropanation and C-H bond insertion. The models which have been developed for the prediction of stereochemistry serve that purpose well. A detailed mechanistic understanding of these processes is desirable to estimate the validity of these models.

REFERENCES

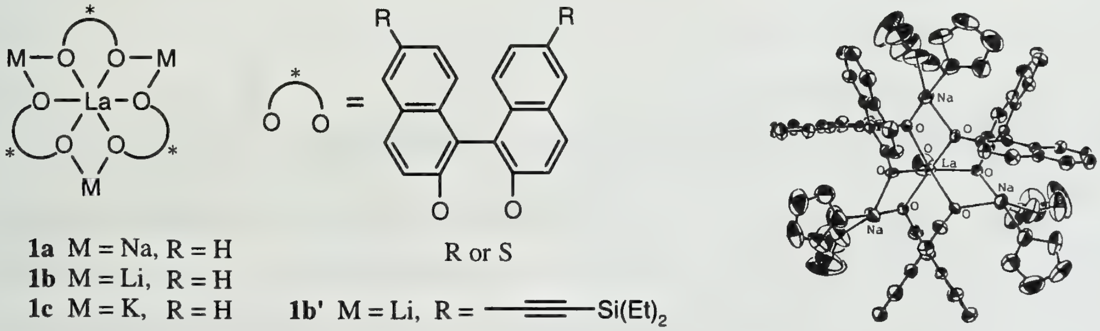
1) Maas, G. *Top. Curr. Chem.* **1987**, *137*, 75.
2) Doyle, M. P. *Chem. Rev.* **1986**, *19*, 348.
3) Doyle, M. P. In Asymmetric Cyclopropanation; Ojima, I., Ed.; VCH Publishers, Inc.: New York, 1993, p 63.
4) Doyle, M. P., Winchester, W. R., Hoorn, J. A. A., Lynch, V., Simonsen, S. H., Ghosh, R. *J. Am. Chem. Soc.* **1993**, *113*, 9968.
5) Padwa, A., Austin, D. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1797.
6) Padwa, A., Austin, D. J., Price, A. T., Semones, M. A., Doyle, M. P., Protopova, M. N., Winchester, W. R., Tran, A. *J. Am. Chem. Soc.* **1993**, *115*, 8669.
7) Brunner, H., Kluschanzoff, H., Wutz, K. *Bull. Soc. Chim. Belg.* **1989**, *98*, 63.
8) Hashimoto, S., Watanabe, N., Sato, T., Shiro, M., Ikegami, S. *Tetrahedron Lett.* **1993**, *34*, 5109.
9) Hashimoto, S., Watanabe, Ikegami, S. *SynLett* **1994**, 1031.
10) Hashimoto, S., Watanabe, N., Ikegami, S. *Synlett* **1994**, 353.
11) McKervey, M. A., Kennedy, M., Maguire, A. R., Roos, G. H. *J. Chem. Soc. Chem. Comm.* **1990**, 361.
12) Pirrung, M. C., Zhang, J. *Tetrahedron Lett.* **1992**, *33*, 5987.
13) Doyle, M. P., Zhou, Q. L., Dyatkin, A. B., Ruppar, D. A. *Tetrahedron Lett* **1995**, *36*, 7579.
14) Pirrung, M. C., Morehead, A. T. Jr., *J. Am. Chem. Soc.* **1994**, *116*, 8991.
15) Doyle, M. P., Eismont, M. Y. *J. Org. Chem.* **1992**, *57*, 6103.
16) Salaün, J. *Chem. Rev.* **1989**, *89*, 1247.
17) Davies, H. M. L. *Tetrahedron* **1993**, *49*, 5203.
18) Martin, S. F., Oalmann, C. J., Liras, S. *Tetrahedron* **1993**, *49*, 3521.
19) Doyle, M. P. *Recl. Trav. Chim. Pays-Bas.* **1991**, *110*, 305.
20) Brookhart, M., Liu, Y. *J. Am. Chem. Soc.* **1991**, *113*, 939.
21) Müller, P., Baud, C., Ené, D., Motallebi, S., Doyle, M. P., Brandes, B. D., Dyatkin, A. B., See, M. M. *Helv. Chim. Acta* **1995**, *78*, 459.
22) Doyle, M. P., Zhou, Q. L., Simonsen, S. H., Lynch, V. *Synlett* **1996**, 697.
23) Doyle, M. P., Pieters, R. J., Martin, S. F., Austin, R. E., Oalmann, C. J., Müller, P. *J. Am. Chem. Soc.* **1991**, *113*, 1423.
24) Doyle, M. P., Austin, R. E., Bailey, A. S., Dwyer, M. P., Dyatkin, A. B., Kalinin, A. V., Kwan, M. Y., Liras, S., Olamann, C. J., Pieters, R. J., Protopopova, M. N., Raab, C. E., Roos, G. H., Zhou, Q. L., Martin, S. F. *J. Am. Chem. Soc.* **1995**, *117*, 5763.
25) Taber, D. F., Ruckle, R. E. Jr. *J. Am. Chem. Soc.* **1986**, *108*, 7686.
26) Doyle, M. P., Pieters, R. J., Taunton, J., Pho, H. Q., Padwa, A., Hertzog, D. L., Percedo, L. *J. Org. Chem.* **1991**, *56*, 820.
27) Doyle, M. P., Westrum, L. J., Wolthuis, W. N. E., See, M. M., Boone, W. P., Bagheri, V., Pearson, M. M. *J. Am. Chem. Soc.* **1993**, *115*, 958.
28) Doyle, M. P., Dyatkin, A. B., Roos, G. H. P., Cañas, F., Pierson, D. A., van Basten, A. *J. Am. Chem. Soc.* **1994**, *116*, 4507.
29) Doyle, M. P., Kalinin, A. V., Ene, D. G. *J. Am. Chem. Soc.* **1996**, *118*, 8837.
30) Müller, P., Polleux, P. *Helv. Chim. Acta.* **1994**, *77*, 645.
31) Padwa, A., Weingarten, M. D. *Chem. Rev.* **1996**, *96*, 223.
32) McCarthy, N., McKervey, M. A., Ye, T., McCann, M., Murphy, E., Doyle, M. P. *Tetrahedron Lett.* **1992**, *33*, 5983.

ENANTIOSELECTIVE TRANSFORMATIONS CATALYZED BY NOVEL HETEROBIMETALLIC COMPLEXES: LANTHANUM-M-1,1'-BI-2-NAPHTHOL (M = Li, Na, K)

Reported by Xiao Yu December 5, 1996

INTRODUCTION

The recent development of the multifunctional multimetallic catalyst complex **1** provides a new approach to improving enantioselectivity in a series of catalytic asymmetric transformations.[1-3] Lanthanum-alkali metal-BINOL complex **1** (Figure 1), the Fluka Prize winning reagent of the year 1996, successfully combines the basicity of alkali metal with the oxophilicity and Lewis acid character of lanthanum in an asymmetric environment provided by chiral ligand BINOL. Effective asymmetric catalysis of three different kinds of reactions: La-Na-BINOL (LSB) **1a** in Michael additions, La-Li-BINOL (LLB) **1b** in nitroaldol reactions and La-K-BINOL (LKB) **1c** in hydrophosphonylation has been reported. These reactions will be reviewed in this abstract.



1a M = Na, R = H
1b M = Li, R = H
1c M = K, R = H
1b' M = Li, R = —≡—Si(Et)$_2$

Figure 1. La-M-BINOL and the X-ray crystal structure of **1a**.

STRUCTURE

The catalytic complexes **1a** - **1c** can be prepared conveniently in laboratory.[1-3] The crystal structure of **1a** was elucidated by X-ray analysis and is shown in Figure 1.[3] The central lanthanum atom is coordinated to three molecules of BINOL and one molecule of water. Attempts to obtain an X-ray quality crystals of the corresponding lithium complexes have not been successful. However, the similarity of the fragment patterns in the mass spectra of **1b** with that of **1a** strongly suggests that they have the same structure.[1]

Copyright © 1996 by Xiao Yu

MICHAEL ADDITION WITH LSB (1A)

Catalytic asymmetric Michael addition is an important synthetic method to introduce chirality into molecules. In this regard, 1,3-dicarbonyl compounds are very useful Michael donors. Michael additions using 1,3-dicarbonyl compounds can be generally divided into three types: Type I reactions use prochiral enolate donors to generate a stereogenic center on the Michael donor fragment, while type II reactions use prochiral Michael acceptors and give a stereogenic center on the acceptor fragment. Type III reactions are the combination of type I and type II reactions. They use both prochiral enolate donors and prochiral receptors to produce two stereogenic centers in one step. These are shown in Figure 2.

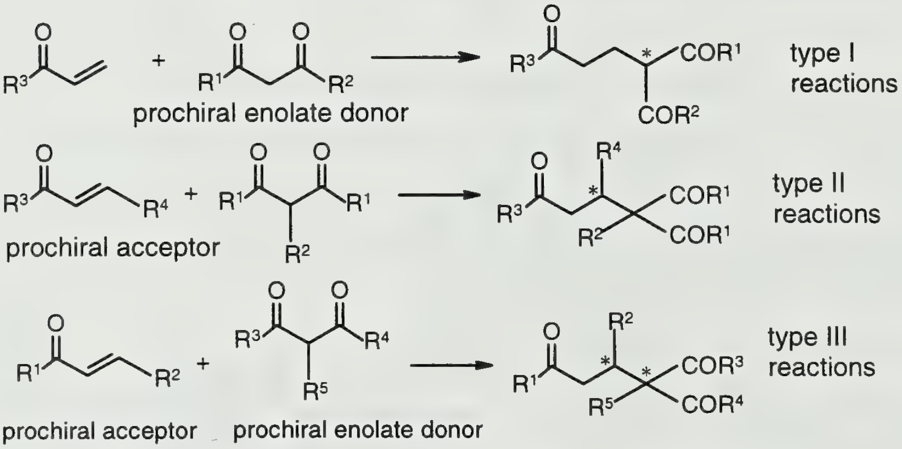


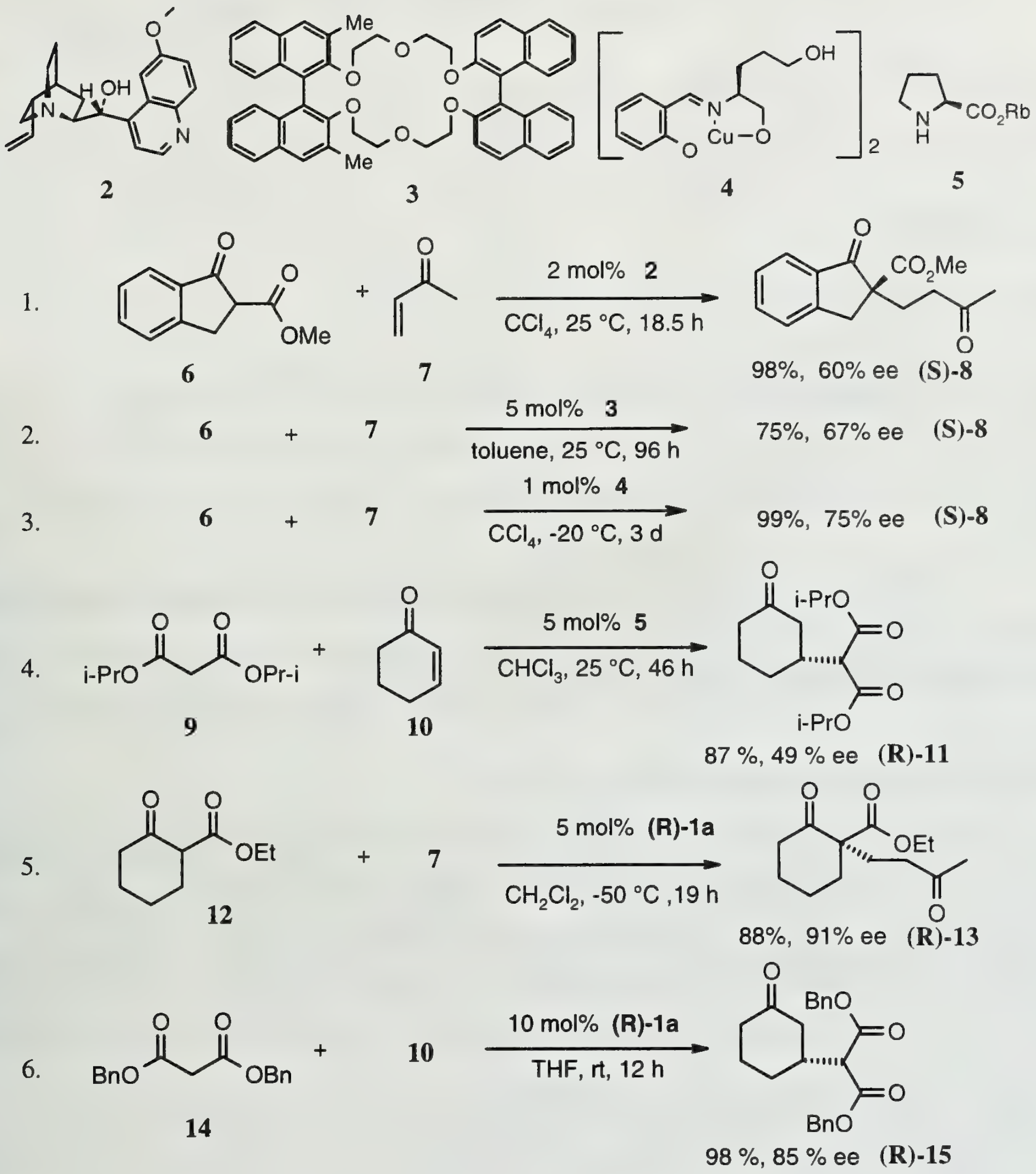
Figure 2 Classification of catalytic asymmetric Michael additions

For type I reactions, optically active chiral pool alkaloids such as quinine, quinidine and cinchona have been studied (reaction 1).[4] Potassium-chiral crown ether complexes (reaction 2)[5] and many other chiral metal complexes such as $Co(acac)_2$ in the presence of chiral ligand diamines,[6] Cu(II) complexes of chiral Schiff bases (reaction 3),[7] Ni(II) complexes of proline derivatives[8] and $Eu(tfc)_3$,[9] have also been used. However, the range of these catalysts is limited to type I reactions, and only moderate (40 - 60 %) *ee* was observed except for chiral crown ethers (up to 80 % *ee*). Compared to type I reactions, there is only a few examples about type II reactions. Proline and its derivatives can provide moderate *ee* in reacton 4.[10,11]

The complex **1a** is a general base catalyst (ONa) and has Lewis acid character (La). It has an ability to coordinate both enolates and Michael acceptor at the same time. As shown in Scheme 1, the complex **1a** promotes both reactions 5 (type I) and reaction 6 (type II) in high *ee* and in good yield.

An ^1H NMR study of reaction 6 in Scheme 1 suggested that the carbonyl group of enone **9** was coordinated to the central lanthanum in **1a**. Compared with the alkali metal

Scheme 1.



free lanthanum BINOL catalyst,[12] the Michael additions catalyzed by the complex **1a** were found to proceed smoothly and with high enantioselectivities even at room temperature. This result illustrates to an important role of bimetallic character in the complex **1a**.

It was also found that nonpolar solvents, such as toluene or CH_2Cl_2, are essential for reaction 5 to obtain high enantioselectivity.[13] This result is rationalized by the fact that in more polar solvents, such as THF, the sodium enolate can dissociate easily from the **1a**.

Shibasaki proposed the catalytic cycle for reaction 6 as shown in Figure 3.[3] The sodium enolate is generated from dimethyl malonate by **1a** and the carbonyl group of enone **10** is coordinated to the central lanthanum so that the two activated reactants are directed

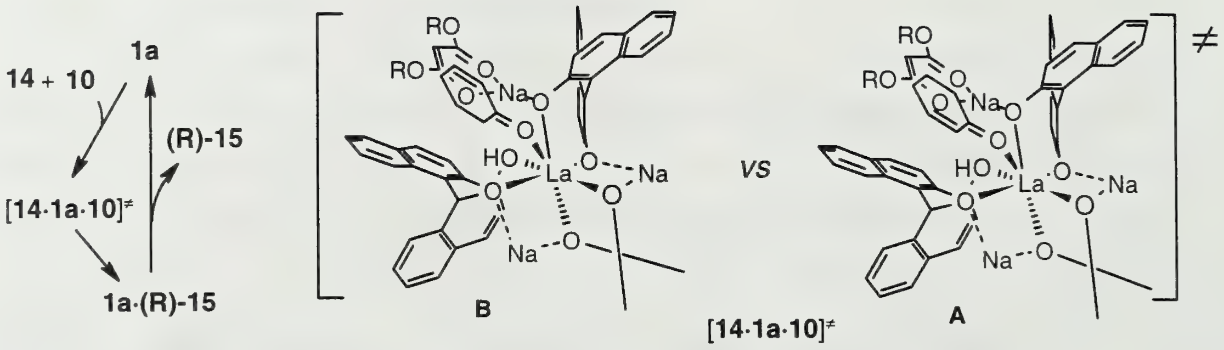


Figure 3. Proposed catalytic cycle of reaction 5.

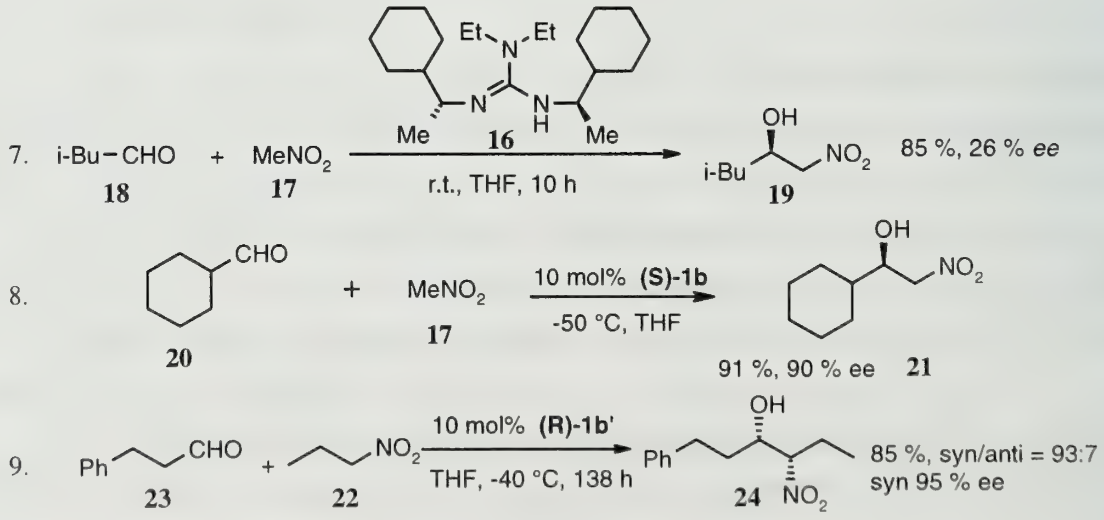
towards each other in the microscopic chiral environment provided by BINOL to give the (R)-adduct stereoselectively. The computational simulation of the transition state by using Rappé's universal force field (UFF) suggests that $[\mathbf{A}]^{\neq}$ is favored by 4.9 kcal/mol in energy over $[\mathbf{B}]^{\neq}$.

In summary, the complex **1a** shows both basicity and Lewis acid character at the same time, which results in high enantioselectivity in Michael reactions of both type I and type II. This is the first reported multifunctional hetero-bimetallic asymmetric catalyst.[3]

NITROALDOL REACTION WITH LLB (1B)

The nitroaldol reaction is a useful C-C bond forming method in organic synthesis. Reduction of the resulting β-nitroalcohols from nitroaldol reactions leads to the corresponding aminoalcohols, a number of which are important chiral auxiliaries.[14] The only alternative asymmetric catalyst studied in nitroaldol reactions is chiral guanidine

Scheme 2.



system, however, the *ee* was very low (reaction 7).[15,16] In comparison, the complexes **1b** and **1b'** give much higher enantioselectivities (reaction 8,9) and **1b'** also shows high diastereoselectivity (reaction 9).[1,17]

The proposed mechanism for the reaction 8 is shown in Figure 4. Both the rare earth nitronate formed and the carbonyl oxygen of the aldehyde **20** are coordinated to the lanthanum atom to generate **21**. The transition state [**E**]$^{\neq}$ is believed to be favored over [**F**]$^{\neq}$ because of the less steric hindrance.

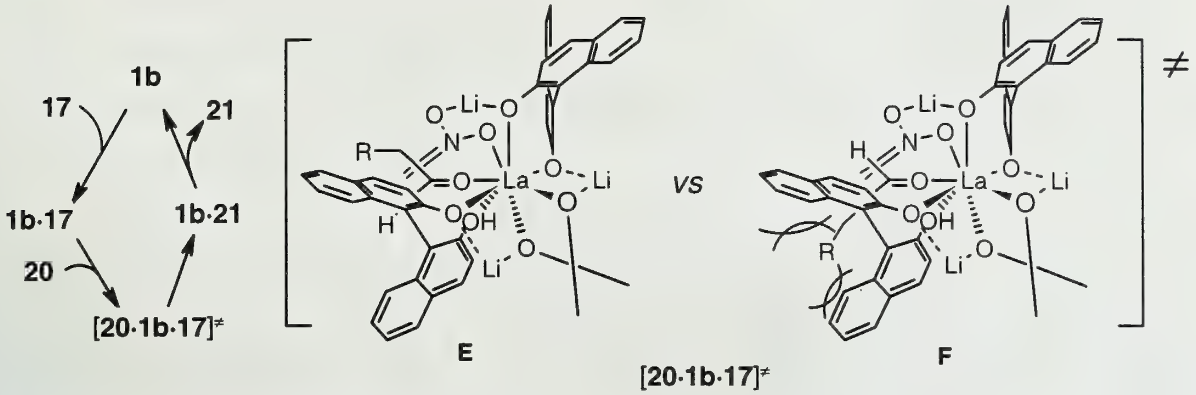
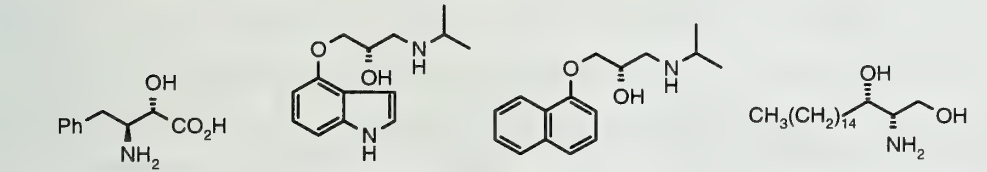


Figure 4. Proposed catalytic cycle for reaction 6.



(2S,3S)-phenylnorstatine **25**

(S)-(-)-pindolol **26**

(S)-(-)-propranolol **27**

threo-dihydrosphingosine **28**

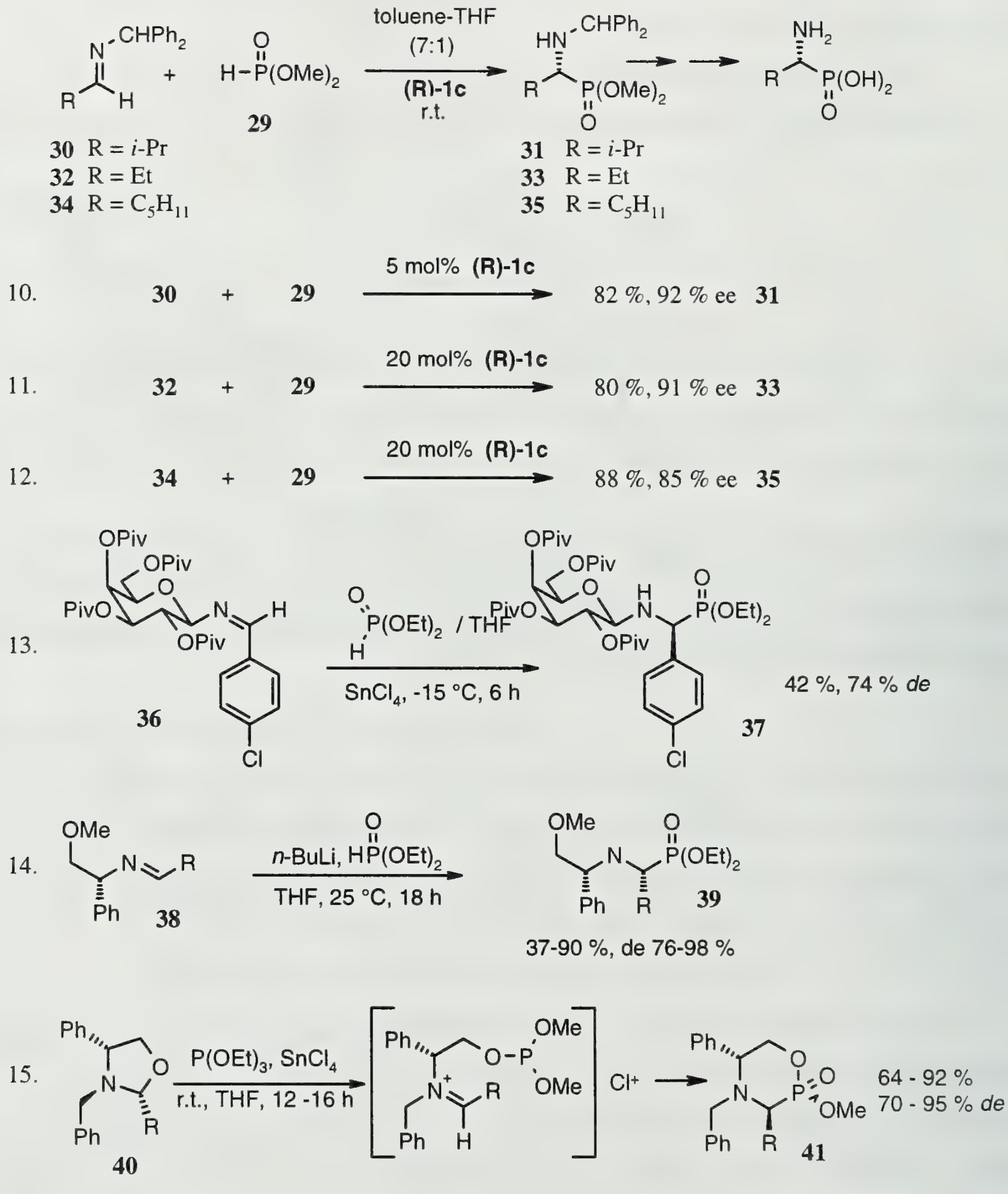
Figure 5. Molecules synthesized by nitroaldol reactions promoted by 1b.

The complexes **1b** and **1b'** have been successfully applied in the syntheses of phenylnorstatine **25**, pindolol **26** propranolol **27** and dihydrosphingosine **28** (Figure 5).[17-20] Compared to the alternative methods,[21-24] the complexes **1b** and **1b'** gave higher yield and higher enantioselectivity in lower cost from inexpensive achiral starting materials by straight-forward synthetic paths.

HYDROPHOSPHONYLATIONS WITH LPB (1C)

Because of their structural analogy to α-amino acids, α-amino phosphonic acids are interesting molecules for the design of phosphonamide-containing peptide proteinase inhibitors. The nucleophilic addition of phosphite to an imine has been broadly used for the

Scheme 3.



asymmetric synthesis of α-amino phosphonic acids because of its simplicity. The complex **1c** was the only asymmetric catalyst reported in this area (reaction 10 - 12)[2].

Alternative asymmetric synthesis of α-amino phosphonic acids involve chiral inducing reagents. A satisfactory result has been achieved by using Schiff bases derived from pivaloylated glycosylamines (reaction 13).[25]. In order to provide a more rigid transition state to improve the *de* further, a chelation strategy[26] and intramolecular reactions[27] have been applied, and higher *de* was obtained (reaction 14, 15). A chiral phosphite has also been studied, however, the *de* is much lower.[28] Compared to these methods, the use of **1c** as a catalyst provides an alternative route to high *ee*.

DISCUSSION

It is interesting to note that **1a**, **1b** and **1c** complement each other in their ability to catalyze asymmetric reactions: **1b** shows high enantioselectivity in nitroaldol reactions in spite of ineffectiveness in Michael addition; while **1a** produces almost racemic Michael adducts, but gives high *ee* products in nitroaldol reactions; and **1c** is particularly effect in asymmetric hydrophosphonylation (Table 1).

Table 1. Comparison of **1a 1b** and **1c** in different reactions.

	1a	**1b**	**1c**
Michael Additions	high	racemic	moderate
Nitroaldol Reactions	low	high	N/A
Hydrophosphonylation	moderate	low	high

The reasons of the markedly different performance of the catalysts have not been clearly rationalized. One possible explanation is based on the structure of the catalyst complexes. As shown in Figure 1, three BINOL ligands surround the central La^{3+} ion. Thus, in order to reach the central La^{3+} ion and bind it, a reactant has to pass through a "window". The radius of Li^+ is smaller than that of Na^+ which makes the "window" in **1b** smaller than that in **1a**, so that for Michael addition (reaction 5), enone **9** can pass through the "window" in **1a**, but can not do so in **1b**. As support for this speculation, ^{1}H-NMR studies do not show a complex of enone with La in **1b** during the reaction. Decreasing the radius of the central lanthanoid atom would have the same type of effect. Studies on using Ln-Na-BINOL (Ln=lanthanoids) as catalysts in Michael reactions demonstrated that as the radius of the central lanthanide decreased, the enantioselectivity also decreased. In the case of europium-Na-BINOL complex, which gave almost racemic Michael adducts, the ^{1}H-NMR spectra indicated that no complex of enone-Eu was formed.[3]

CONCLUSION

Convenient preparation and high enantioselectivity makes **1a**, **1b** and **1c** superior to the alternative asymmetric catalysts for Michael addition, nitroaldol reaction and hydrophosphonylation.

REFERENCES

1) Sasai, H.; Suzuki, T.; Itoh, N.; Tanaka, K.; Date, T.; Okamura, K.; Shibasaki, M. *J. Am. Chem. Soc.* **1993**, *115*, 10372-10373.
2) Sasai, H.; Arai, S.; Tahara, Y.; Shibasaki, M. *J. Org. Chem.* **1995**, *60*, 6656-6657.
3) Sasai, H.; Arai, T.; Satow, Y.; Houk, K. N.; Shibasaki, M. *J. Am. Chem. Soc.* **1995**, *117*, 6194-6198.
4) Hermann, K.; Wynberg, H. *J. Org. Chem.* **1979**, *44*, 2238.
5) Cram, D. J.; Sogah, G. D. Y. *J. Chem. Soc., Chem. Commun.* **1981**, 625.
6) Brunner, H.; Hammer, B. *Angew. Chem. Int. Ed. Engl.* **1984**, *23*, 312-313.
7) Desimoni, G.; Dusi, G.; Faita, G.; Quadrelli, P.; Righetti, P. *Tetrahedron* **1995**, *51*, 4131-4144.
8) Botteghi, C.; Paganelli, S.; Schionato, A.; Boga, C.; Fava, A. *J. Mol. Catal.* **1991**, *66*, 7.
9) Bonadies, F.; Lattanzi, A.; Orelli, L. R.; Pesci, S.; Scettri, A. *Tetrahedron Lett.* **1993**, *34*, 7649-7650.
10) Yamaguchi, M.; Shiraishi, T.; Hirama, M. *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1176-1178.
11) Yamaguchi, M.; Shiraishi, T.; Igarashi, Y.; Hirama, M. *Tetrahedron Lett.* **1994**, *35*, 8233-8236.
12) Sasai, H.; Arai, T.; Shibasaki, M. *J. Am. Chem. Soc.* **1994**, *116*, 1571-1572.
13) Sasai, H.; Emori, E.; Arai, T.; Shibasaki, M. *Tetrahedron Lett.* **1996**, *37*, 5561-5564.
14) Ager, D. J.; Prakash, I.; Schaad, D. R. *Chem. Rev.* **1996**, *96*, 835.
15) Chinchilla, R.; Nájera, C.; Sánchez-Agulló, P. *Tetrahedron: Asymmetry* **1994**, *5*, 1393-1402.
16) Davis, A. P.; Dempsey, K. J. *Tetrahedron: Asymmetry* **1995**, *6*, 2829-2840.
17) Sasai, H.; Tokunaga, T.; Watanabe, S.; Suzuki, T.; Itoh, N.; Shibasaki, M. *J. Org. Chem.* **1995**, *60*, 7388-7389.
18) Sasai, H.; Yamada, Y. M. A.; Suzuki, T.; Shibasaki, M. *Tetrahedron* **1994**, *50*, 12313-12318.
19) Sasai, H.; Kim, W. S.; Suzuki, T.; Shibasaki, M.; Mitsuda, M.; Hasegawa, J.; Ohashi, T. *Tetrahedron Lett.* **1994**, *35*, 6123-6126.
20) Sasai, H.; Suzuki, T.; Itoh, N.; Shibasaki, M. *Appl. Organomet. Chem.* **1995**, *9*, 421-426.
21) Troxler, F.; Hofmann, A. *Chem. Abstr.* **1970**, *72*, 66806j.
22) Reetz, M. T.; Drewes, M. W.; Harms, K.; Reif, W. *Tetrahedron Lett.* **1988**, *29*, 3295-3298.
23) Shibuya, H.; Kawashima, K.; Narita, N.; Ikeda, M.; Kitagawa, I. *Chem. Pharm. Bull* **1992**, *40*, 1154-1165.
24) Klunder, J. M.; Ko, S. Y.; Sharpless, K. B. *J. Org. Chem.* **1986**, *51*, 3710.
25) Laschat, S.; Kunz, H. *Synthesis* **1992**, 90.
26) Smith, A. B. I.; Yager, K. M.; Taylor, C. M. *J. Am. Chem. Soc.* **1995**, *117*, 10879-10888.
27) Maury, C.; Gharbaoui, T.; Royer, J.; Husson, H. *J. Org. Chem.* **1996**, *61*, 3687-3693.
28) Hoppe, I.; Schöllkopf, V.; Nieger, M.; Egeret, E. *Agnew. Chem.* **1985**, *97*, 1066.

MOLECULAR IMPRINTING–A PRACTICAL TOOL OR NOVEL CURIOSITY?

Reported by Ned Zimmerman 12 December 1996

INTRODUCTION

Molecular imprinting[1-3] has been proposed as a novel method for achieving selective recognition of substrates in non-biological systems. Molecular imprinting typically involves the incorporation of a template molecule into a mixture of functional monomer, cross-linking monomer, radical initiator, and solvent. Polymerization and template removal yield a rigid polymer with cavities complementary to the template (see Figure 1). The approach has been suggested for application in a variety of fields including chromatography,[4-18] catalysis,[19-25] immunoassay,[26-28] and sensor technology.[29-34] Molecular imprinting in polymers was first reported in 1972 by Wulff and Sarhan who successfully imprinted D-glyceric acid in divinylbenzene.[35] Wulff and Sarhan established that their imprinted divinylbenzene was able to distinguish between the racemates of both glyceric acid and glyceraldehyde in equilibrium batch separations. Since then many other compounds, including amino acids and peptides, carbohydrates, drugs, steroids, and herbicides, have been investigated as templates in molecularly imprinted polymers (MIPs). This review will focus on the application of MIPs to chromatography, catalysis, and radiolabelled ligand-binding assays.

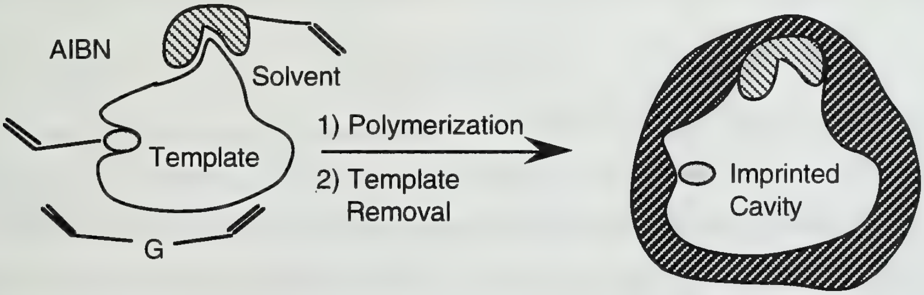


Figure 1. The molecular imprinting process. The template is associated with generic functional monomers having olefinic functionality. A generic cross-linking agent is also present.

BACKGROUND

The mechanism of molecular imprinting is often considered in analogy to enzymes and antibodies. MIPs could potentially mimic enzymes through a combination of functional group and molecular shape recognition. Molecular shape recognition results from a highly cross-linked

Copyright © 1996 by Ned Zimmerman

polymer molding itself around the template. Functional group recognition is induced by the selective orientation of functional groups complementary to those of the template during polymerization. Two methods of induction are practiced. In the first, the functional monomer and template are covalently linked prior to polymerization. After polymerization and template removal, it is hoped that the exposed functional groups are positioned to covalently bind a properly oriented substrate in the cavity. This method has been extensively investigated by Wulff and coworkers, mostly with vinylbenzeneboronate diesters to template diols.[17,36,37] While this method can lead to cavities with a well defined functional group orientation, the rate of recognition is limited by the kinetics of the formation and dissociation of the covalent bonds.[17,38]

In the second approach, noncovalent interactions are utilized to orient the functional monomers around the template. Hydrogen bond and ionic interactions have been shown to be the most important.[39] This approach requires a large excess of functional monomer to insure that the template molecules are undergoing the maximum number of interactions at the time of polymerization.[2] In addition, apolar solvents are used to maximize the strength of the noncovalent interactions during polymerization. The solvent used also affects the properties of the polymer including pore size, interior surface area, and swelling. Optimal polymer properties are obtained with polar solvents and high temperatures.[39] In contrast, noncovalent interactions are maximized at low temperatures.[39] Because of the excess functional monomer, the polymers possess cavities with a wide range of binding affinities. In subsequent use of the polymer the weak, noncovalent interactions can lead to non-specific binding of the substrate, limiting resolution.[40]

MECHANISM

In order to rationally optimize the recognition and binding properties of MIPs for specific templates, an understanding of the mechanism of recognition is necessary. While an accurate model has not yet been developed, important factors have been identified and investigated.[9,14,38,39,41,42] The most important element of recognition is the functional group complementarity between the template and the MIP. Because most MIPs use hydrogen bonding and ionic interactions to define the spatial array of functional groups in the cavities, polar, protic solvents may disrupt recognition both during polymerization and in subsequent binding of substrate.[43] Thus a methacrylic acid (MAA) and ethyleneglycoldimethacrylate (EDMA) copolymer prepared in acetic acid with a L-phenylalanine anilide (L-PheNHPh) template displayed moderate separation of D,L-PheNHPh in chiral chromatography ($\alpha = 1.9$), while the corresponding polymer prepared in CH_2Cl_2 showed significant separation ($\alpha = 8.2$).[39] The strength of the ionic interaction can be enhanced by incorporating functional monomers of greater basicity when acidic templates are used.[42] The larger ΔpK_a between template and monomer enhances proton transfer and charge localization, thus increasing the strength of the ionic interaction.

Another factor affecting the recognition mechanism is the substrate shape. The role shape plays in the recognition mechanism varies from system to system. Shape relies upon weaker non-bonded interactions which are easier to disrupt than hydrogen bonds or ionic interactions. This is illustrated by the decreased recognition efficiences of MIPs in protic solvents. Larger molecules with rigid structures present greater surface areas and exhibit better shape selectivity.[19,44] For example, Byström and coworkers observed complete control over the reduction of a steroid-3,17-diketone at either the 3 or 17 position depending upon the choice of template.[19] The selectivity in this reaction is dictated entirely by shape, no other functional interactions are present. Shape also relies upon the polymer molding tightly around the template. As expected, polymers with low degrees of cross-linking, or excessive swelling, do not provide good separation.

For templates which have multiple points of interaction with the polymer, the selectivity observed is the result of a cooperative binding effect. The first binding event must be rate determining with respect to a rapid second binding event.[38] If the reverse is true, selective binding would not occur under non-equilibrium conditions. Substrates capable of two point binding are retained longer in the cavity because of the additional stabilization from the second binding site. The increased binding affinity is especially pronounced with covalent linkages such as boronate esters[36] or ketals.[38] The separation is based upon the rates of dissociation of the different substrates. Substrates that do not have the proper orientation of functional groups will only exhibit one point binding, and dissociate faster. Cooperative binding relies upon an interplay of functional group orientation and interaction in the cavity to achieve selectivity. Increased selectivity should be observed for templates with a larger number of binding sites.

APPLICATIONS OF MOLECULAR IMPRINTING

Chromatography

Molecular imprinting has been applied to several chromatographic systems[45] including TLC,[46], capillary electrophoresis,[47] and analytical HPLC. Considerable attention has been given to chiral chromatography, although MIPs have also been used to separate bis-imidazoles,[48,49] xanthines,[13] herbicides,[14,50] and nucleotides.[18] Chiral chromatography in MIPs is distinctive because the order of elution of the antipodes can be predicted, the most retained antipode is the imprint species. Other chiral stationary phases (CSPs) do not provide this information *a priori*.[45] In its simplest form an equilibrium batch separation selectively binds one enantiomer leaving the other in solution. Wulff and coworkers observed a 40.4 % enrichment of the polymer bound enantiomer by this method.[37]

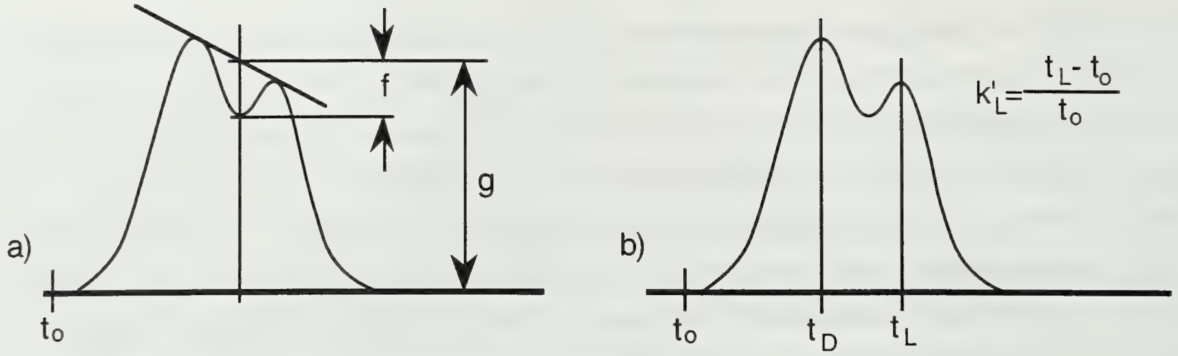


Figure 2. a) Definition of f/g. b) Definition of k'.

Considerable attention has been given to developing MIPs for chiral resolution by analytical HPLC. Chiral resolution has been achieved on a number of substrates including carbohydrates, amino acid derivatives, peptides, drugs, and β-blockers. A column's ability to resolve enantiomers is measured in three different ways: the separation factor, α ($\alpha = k'_L/k'_D$ where $k'_L = (t_L-t_o)/t_o$. See Figure 2 b.); the resolution, $R_s = \Delta t_r/w_{av}$ (w_{av} = average peak width at the baseline), which takes into account the peak overlap and tailing; and Kaiser's resolution, f/g (defined in Figure 2 a).[51] The value most often reported is α. Good separation is obtained for $\alpha > 2$; an $\alpha > 3$ usually indicates baseline resolution. Much of the work in MIPs has been devoted to optimizing the separation factor, α.

Several problems have been addressed in the optimization of α for different templates. The heterogeneity of recognition sites leads to poor separation, tailing, and severe peak broadening for the retained antipode. Gradient elution has been used to eliminate the peak broadening.[9,52] Matsui and coworkers addressed the site heterogeneity by polymerizing the MIPs in the column to create a rod type MIP. An $\alpha = 1.4$ was obtained, and tailing and peak broadening were significantly reduced.[13] Sellergren reported a dispersion polymerization *in situ* with higher αs (2.2-7.9), but much broader peaks.[16] Dispersion polymerization leads to MIPs of homogeneous particle size. These columns have greater efficiency, a higher number of theoretical plates, and better flow rates. A direct comparison of D,L-PheNHPh analysis on dispersion and bulk MIPs shows less separation ($\alpha = 2.2$ vs. $\alpha = 3.5$[39]) for the dispersion MIP. This is probably due to the polar polymerization solvent and a low functional monomer concentration.[16] Both the rod type and dispersion MIPs could affect substrate resolution with aqueous mobile phases.[13,16] This probably reflects good substrate access to high affinity binding sites. Recognition is achieved through solvent exclusion upon substrate binding, similar to enzymatic systems. The polymer assists solvent exclusion because of its unfavorable interactions with the mobile phase.

In an alternate dispersion polymerization, uniformly sized particles of poly(trimethylolpropane trimethacrylate) (TRIM) containing 8-10 % unreacted methacrylate groups were made. The pore system was then imprinted with MAA and template. Separations of Boc-Phe-OH with $\alpha = 1.24$-1.74 and a column efficiency about twice that of the bulk MIP column were obtained.[6] Increased flow rates were also observed. Similar preparations of MIPs on methacrylate functionalized silica gel particles have been used to separate proteins[9] and bis-imidazoles,[15] in the latter case with $\alpha > 1.4$. This represents an important extension of molecular imprinting towards chromatographic protein separation. It represents a practical, non-biological, process for selective protein isolation.

TRIM has also been used to enhance the cross-linking in conventional bulk MIPs. The polymers demonstrated good separation ($\alpha = 1.92$, f/g = 0.92) with high load capacity (1 mg substrate/ 1.6 g of polymer) for a mixture of Cbz-Ala-Ala-OMe substrates.[52] A related cross-linking monomer, pentaerythritol triacrylate (PETRA) displayed comparable resolution ($\alpha = 2.06$, f/g = 0.93) and load capacity (1 mg) for Cbz-D,L-Tyr-OH substrates.[10] Improved chromatographic properties are the result of the higher degree of cross-linking in TRIM MIPs. The greater structural rigidity reduces MIP swelling, leaving more well defined binding sites. Higher load capacities are the result of greater site homogeneity which reflects an increase in the number of high affinity binding sites in the polymer (the sites responsible for separation). Further advances in chiral chromatography with MIPs will require additional gains in optimizing load capacity, flow rate, and binding site homogeneity.

Catalysis

Many different catalytic reactions in MIPs have been investigated including ester hydrolyses,[22,24,25] hydride reductions,[19,21] eliminations,[20] and aldol condensations.[23] Enantioselective catalysis has been investigated through selective binding of either the ground state[19,22] or the transition state analogues.[21,25] In either case, the enantiomeric excesses are still quite low, generally less than 66% ee. The rhodium system reported by Lemaire and coworkers has demonstrated the best selectivity. The selectivity results from both the chiral diamine ligand bound to the rhodium, and the chiral template which is a transition state analogue. Under optimized conditions the templated catalyst affords 18-19% greater ee than the polymer catalyst without template. Unfortunately, this represents only an 11% increase in ee over the corresponding homogeneous reaction. Both homogenous and polymer supported reactions provide yields of 91 % - 100 %. It was thought that the defined microenvironment of the imprinted cavity would significantly increase the enantioselectivity of the reaction. The results suggest that the catalytic sites are open and not well defined.

The catalytic aldol condensation of acetophenone with benzaldehyde to form chalcone is also of interest because it involves carbon-carbon bond formation.[23] Mosbach and coworkers found an eight fold enhancement over the solution reaction when they imprinted dibenzoylmethane into a divinylbenzene-styrene copolymer using vinyl pyridine complexed to Co^{2+} as the functional monomer. The rate enhancement may be the result of localization of the reactants in the presence of Lewis acidic Co^{2+}. The MIP also demonstrated substrate selectivity against adamantyl methyl ketone and 9-acetylanthracene. The steric bulk of these compounds would preclude their incorporation into the active site. These results demonstrate that MIPs can catalyze carbon-carbon bond forming reactions under harsh conditions (DMF, 100 °C, several days) with the polymer retaining 80-95 % of its activity upon re-use.[23] In contrast, enzymatic systems could not survive the reaction conditions.

Immunoassays

Recent efforts have demonstrated the utility of molecularly imprinted antibody mimics for radiolabelled ligand-binding assays.[26-28] Theophylline and diazepam imprinted polymers were used to assay human blood serum for these drugs.[28] Mosbach and coworkers found that the molecularly imprinted sorbent assay (MIA) for theophylline was linear over the range of 14-224 μM under optimized conditions. The corresponding MIA for diazepam was linear over the range of 0.44-28 μM. Cross-reactivity for both MIAs was comparable to the corresponding antibody assays. Limits of detection were 3.5 μM and 0.2 μM respectively. Mosbach and coworkers also studied the binding properties of morphine and Leu-enkephalin MIPs.[27] Low cross-reactivity was observed for both MIPs in organic solvents and the morphine MIP even showed good selectivity in an aqueous buffer. Multiple K_ds were observed by Scatchard plot analysis indicating the heterogeneity of the binding sites. Andersson recently reported an optimization of the aqueous buffer assay on (S)-propanolol.[26] He observed a six nanomolar limit of detection in aqueous buffer containing 2 % ethanol with a cross-reactivity of less than 1.5 % for the enantiomer. The calibration curve had a linear range of 10^1-10^5 nM, making it potentially useful in pharmacokinetic studies.

CONCLUSION

Molecular imprinting is a novel method for the selective recognition of substrates. The potential exists for the preparative resolution of chiral compounds, the asymmetric catalysis of reactions, and the selective separation of proteins using non-biological media. However, much work remains to be done before the practical application of molecular imprinting to chromatography and catalysis can be realized. A better understanding of the mechanism of selective recognition, and efforts to optimize the polymer properties and to increase binding site homogeneity are needed. This is a daunting task. Molecular level details are extremely difficult to investigate in amorphous polymers, a problem that will continue to hinder mechanistic investigations. Optimization of polymer properties is difficult because noncovalent interactions are optimal under a different set of conditions. The limiting factor may be the polymer morphology. A polymer that is dense enough to afford a homogeneous set of well defined cavities cannot remain porous enough to allow substrate and solvent to pass through the polymer. This analysis suggests that molecular imprinting will likely remain a novel curiosity.

REFERENCES

1) Mosbach, K. *Trends Biochem. Sci.* **1994**, *19*, 9-14.
2) Wulff, G. *Angew. Chem. Int. Engl.* **1995**, *34*, 1812-1832.
3) Shea, K. J. *Trends Polym. Sci.* **1994**, *2*, 166-173.
4) Andersson, L. I.; Muller, R.; Mosbach, K. *Macromol. Rap. Comm.* **1996**, *17*, 65-71.
5) Dhal, P. K.; Vidyasankar, S.; Arnold, F. H. *Chem. Mater.* **1995**, *7*, 154-152.
6) Glad, M.; Reinholdsson, P.; Mosbach, K. *Reactive Polym.* **1995**, *25*, 47-54.
7) Hosoya, K.; Yoshizako, K.; Shirasu, Y.; Kimata, K.; Araki, T.; Tanaka, N.; Haginaka, J. *J. Chromatogr. A* **1996**, *728*, 139-147.
8) Kempe, M.; Mosbach, K. *J. Chromatogr. A* **1994**, *664*, 276-279.
9) Kempe, M.; Mosbach, K. *J. Chromatogr. A* **1995**, *691*, 317-323.
10) Kempe, M. *Anal. Chem.* **1996**, *68*, 1948-1953.
11) Kugimiya, A.; Takeuchi, T.; Matsui, J.; Ikebukuro, K.; Yano, K.; Karube, I. *Anal. Lett.* **1996**, 1099-1107.
12) Mallik, S.; Plunkett, S. D.; Dhal, P. K.; Johnson, R. D.; Pack, D.; Shnek, D.; Arnold, F. H. *New J. Chem.* **1994**, *18*, 299-304.
13) Matsui, J.; Miyoshi, Y.; Matsui, R.; Takeuchi, T. *Anal. Sciences* **1995**, *11*, 1017-1019.
14) Matsui, J.; Miyoshi, Y.; Doblhoffdier, O.; Takeuchi, T. *Anal. Chem.* **1995**, *67*, 4404-4408.
15) Plunkett, S. D.; Arnold, F. H. *J. Chromatogr. A* **1995**, *708*, 19-29.
16) Sellergren, B. *J. Chromatogr. A* **1994**, *673*, 133-141.
17) Wulff, G.; Poll, H.-G.; Minárik, M. *J. Liq. Chrom.* **1986**, *2 & 3*, 385-405.
18) Shea, K. J.; Spivak, D. A.; Sellergren, B. *J. Am. Chem. Soc.* **1993**, *115*, 3368-3369.
19) Byström, S. E.; Börje, A.; Akermark, B. *J. Am. Chem. Soc.* **1993**, *115*, 2081-2083.
20) Beach, J. V.; Shea, K. J. *J. Am. Chem. Soc.* **1994**, *116*, 379-380.
21) Gamez, P.; Dunjic, B.; Pinel, C.; Lemaire, M. *Tetrahedron Lett.* **1995**, *36*, 8779-8782.
22) Morihara, K.; Takiguchi, M.; Shimada, T. *Bull. Chem. Soc. Japan* **1994**, *67*, 1078-1084.
23) Matsui, J.; Nicholls, I. A.; Karube, I.; Mosbach, K. *J. Org. Chem.* **1996**, *61*, 5414-5417.
24) Ohkubo, K.; Urata, Y.; Hirota, S.; Funakoshi, Y.; Sagawa, T.; Usui, S.; Yoshinaga, K. *J. Molec. Catalysis-a-Chemical* **1995**, *101*, L111-L114.
25) Sellergren, B.; Shea, K. J. *Tetrahedron: Asym.* **1994**, *5*, 1403-1406.
26) Andersson, L. I. *Anal. Chem.* **1996**, *68*, 111-117.
27) Andersson, L. I.; Müller, R.; Vlatakis, G.; Mosbach, K. *Proc. Nat. Acad. Sci.* **1995**, *92*, 4788-4792.
28) Vlatakis, G.; Andersson, L. I.; Müller, R.; Mosbach, K. *Nature* **1993**, *361*, 645-647.
29) Piletsky, S. A.; Parhometz, Y. P.; Lavryk, N. V.; Panasyuk, T. L.; El'skaya, A. V. *Sensors and Actuators B* **1994**, *18-19*, 629-631.
30) Piletsky, S. A.; Piletskaya, K.; Piletskaya, E. V.; Yano, K.; Kugimiya, A.; Elgersma, A. V.; Levi, R.; Kahlow, U.; Takeuchi, T.; Karube, I.; Panasyuk, T. I.; Elskaya, A. V. *Anal. Lett.* **1996**, 157-170.
31) Hedborg, E.; Winquist, F.; Lundström, I.; Andersson, L. I.; Mosbach, K. *Sensors and Actuators A* **1993**, *37-38*, 796-799.
32) Starodub, F. F.; Piletsky, S. A.; Lavryk, N. V.; El'skaya, A. V. *Sensors and Actuators B* **1993**, *13-14*, 708-710.
33) Yoshikawa, M.; Izumi, J.; Kitao, T.; Koya, S.; Sakamoto, S. *J. Membrane Science* **1995**, *108*, 171-175.
34) Kriz, D.; Mosbach, K. *Anal. Chim. Acta* **1995**, *300*, 71-75.
35) Wulff, G.; Sarhan, A. *Angew. Chem. Int. Engl.* **1972**, *11*, 341.
36) Wulff, G.; Dederichs, W.; Grotstollen, R.; Jupe, C. *Affinty Chrom. Rel. Techniques* **1982**, 207-210.
37) Wulff, G.; Schauhoff, S. *J. Org. Chem.* **1991**, *56*, 395-400.
38) Shea, K. J.; Sasaki, D. Y. *J. Am. Chem. Soc.* **1991**, *113*, 4109-4120.

39) Sellergren, B.; Shea, K. J. *J. Chromatogr.* **1993**, *635*, 31-49.
40) Ramström, O.; Nicholls, I. A.; Mosbach, K. *Tetrahedron: Asym.* **1994**, *5*, 649-656.
41) Sellergren, B.; Lepistö, M.; Mosbach, K. *J. Am. Chem. Soc.* **1988**, *110*, 5853-5860.
42) Matsui, J.; Kaneko, A.; Miyoshi, Y.; Yokoyama, K.; Tamiya, E.; Takeuchi, T. *Anal. Lett.* **1996**
2071-2078.
43) Nicholls, I. A.; Ramstrom, O.; Mosbach, K. *J. Chromatogr. A* **1995**, *691*, 349-353.
44) Whitcombe, M. J.; Rodriguez, M. E.; Villar, P.; Vulfson, E. N. *J. Am. Chem. Soc.* **1995**, *117*,
7105-7111.
45) Kempe, M.; Mosbach, K. *J. Chromatogr. A* **1995**, *694*, 3-13.
46) Kriz, D.; Kriz, C. B.; Andersson, L. I.; Mosbach, K. *Anal. Chem.* **1994**, *66*, 2636-2639.
47) Nilsson, K.; Lindell, J.; Norrlöw, O.; Sellergren, B. *J. Chromatogr. A* **1994**, *680*, 57-61.
48) Vidyasankar, S.; Dhal, P. K.; Plunkett, S. D.; Arnold, F. H. *Biotech. Bioeng.* **1995**, *48*, 431-436.
49) Dhal, P. K.; Arnold, F. H. *New J. Chem.* **1996**, *20*, 695-698.
50) Matsui, J.; Doblhoffdier, O.; Takeuchi, T. *Chem. Lett.* **1995**, 489-489.
51) Meyer, V. R. *Chromatographia* **1987**, *24*, 639-645.
52) Kempe, M.; Mosbach, K. *Tetrahedron Lett.* **1995**, *36*, 3563-3566.

UNIVERSITY OF ILLINOIS-URBANA
3 0112 031977405

Q.547
I ℓ 6s
1996/97:2

ORGANIC SEMINAR ABSTRACTS
1996-97, SEMESTER II

University of Illinois

Department of Chemistry
Box 68 Roger Adams Laboratory
600 South Matthews Avenue
Urbana, Illinois 61801

June, 1997

Copyright © by the Board of Trustees of the University of Illinois

NOTICE: **Return or renew all Library Materials! The *Minimum* Fee for each Lost Book is $50.00.**

The person charging this material is responsible for its return to the library from which it was withdrawn on or before the **Latest Date** stamped below.

Theft, mutilation, and underlining of books are reasons for disciplinary action and may result in dismissal from the University.
To renew call Telephone Center, 333-8400

UNIVERSITY OF ILLINOIS LIBRARY AT URBANA-CHAMPAIGN

CHEMISTRY LIBRARY

L161—O-1096

LIBRARY U. OF I.

CH

SEMINAR TOPICS

Semester II, 1996-97

Page

Theory and Applications of Two Directional Chain Synthesis............................1
Jason Clay Pearson

Macrolide Polyketide Biosynthesis and Genetically Engineered Novel Polyketide Metabolites 9
Deborah S. Mortensen

Syntheses of Antiviral Carbocyclic Nucleosides..17
Steven Ma

Stereoselective Synthesis of Polysubstituted Olefins by 1,2-Metallate Rearrangement and 1,1-Bimetalloalkenes..25
Rosanna Tedesco

Asymmetric Induction in Palladium-Catalyzed Allylic Substitution Reactions..........33
Daniel J. Pippel

Studies on the Biosynthesis of Paclitaxel...41
Virginia K.B. Lehmann

Vicarious Nucleophilic Substitution...49
Alex Shvets

Synthetic Methods for Catalytic Asymmetric Aziridination................................57
Michael S. Wendland

Chemically Useful Enzymatic Reactions in Organic Solvents...............................65
David J. Schenk

Allylindium Reagents in Organic Synthesis....73
Corey Stanchina

Unnatural Biopolymers....81
Yoonkyung Kim

THEORY AND APPLICATIONS OF TWO DIRECTIONAL CHAIN SYNTHESIS

Reported by Jason Clay Pearson February 6, 1997

INTRODUCTION

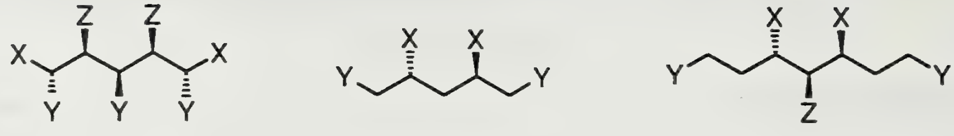
For years, synthetic organic chemists have utilized two basic approaches, linear and convergent, for the synthesis of complex organic molecules. Linear (one directional) synthesis is well suited for short chain synthetic applications but suffers from low material throughput when applied to longer syntheses. Convergent synthesis (one directional) offers a partial solution by allowing increased material throughput but usually requires an equivalent number of synthetic transformations. Some synthetic targets have features that allow a third type of synthesis, two directional chain synthesis. There are two types of two directional synthesis, sequential and simultaneous homologation. Simultaneous homologation involves adding identical groups to each side of a synthetic target. This approach has the advantage of increasing the enantiopurity and decreasing the total number of transformations relative to either a convergent or a linear synthesis. Two directional chain synthesis has existed for a long time but was formalized by Schreiber in 1987.[1] Schreiber divided structures into classes based on the fragment symmetry (Scheme 1). The three major types of symmetry related structures are achiral and meso chains, C_2 symmetric chains, and pseudo C_2 symmetric chains.[1] For this type of strategy to be efficient, group- or face-selective transformations must exist to distinguish the two elongated ends. In some cases, the terminus selective reactions can proceed with unusually high levels of enantiomeric excess,[2] which is an additional benefit. This report will focus on simultaneous homologation and terminus differentiation reactions.

CHAIN SYNTHESIS AND TERMINUS DIFFERENTIATION

Achiral and Meso Chains. These chains are achiral therefore manipulations that result in new stereogenic centers must be controlled by the existing stereochemistry of the substrate (substrate controlled reactions).[1] Chiral reagents cannot be used to elongate the chain because both the substrate and the desired product is achiral. Utilizing chiral reagents with an achiral substrate will give undesired chiral racemic products. There are many examples of two directional chain synthesis of achiral and meso chains.[3]

Copyright © 1997 by Jason Clay Pearson

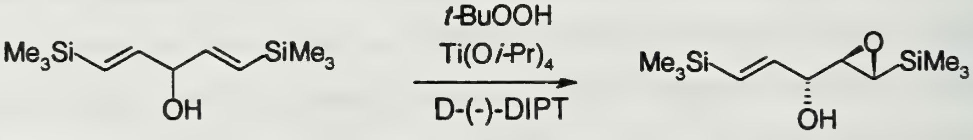
Scheme 1



Achiral C$_2$ Symmetric Pseudo C$_2$ Symmetric

The two directional synthesis of achiral and meso compounds results in molecules with enantiotopic termini. To distinguish the chain ends, reaction with a chiral reagent is required.[1] Chiral reagents provide an additional attractive feature, either enantiomer of the target molecule can usually be formed with very high enantiomeric excess.[2] The most common example in the literature is the selective terminus differentiation of divinyl carbinols using the Sharpless asymmetric epoxidation (over 12 examples).[4] The Sharpless procedure provides access to either enantiomer of the desymmetrized molecule. Kinetic resolution following desymmetrization can be utilized to yield enantiopure products. Scheme 2 provides a clear example of the utility of this reaction. Starting material **1** was prepared from ethyl formate with (*E*)-$Me_3SiCH{=}CHLi$ and then underwent Sharpless asymmetric epoxidation to give **2** in 92% yield and >95% ee.[5]

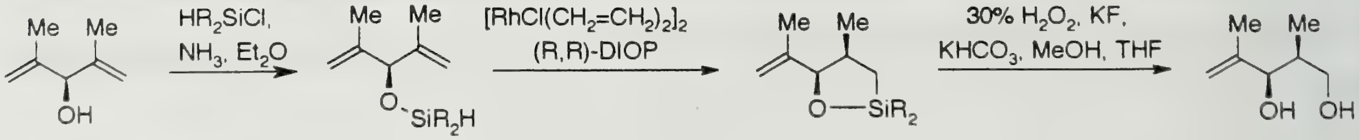
Scheme 2



1 D-(-)-DIPT= diisopropyl tartrate 2

Catalytic asymmetric hydrosilation has also been applied to the terminus differentiation of divinyl carbinols utilizing a rhodium(I) complex with an optically active phosphine ligand, (*R*,*R*)-DIOP (Scheme 3).[6] Divinyl carbinol **3** was converted into the silyloxy derivative **4** which underwent intramolecular hydrosilation in the presence of 2 mol % of the chiral rhodium complex to give **5**. Peroxide oxidation of **5** yielded diol **6** in 80% yield and 87% ee (>99/1 syn/anti).

Scheme 3

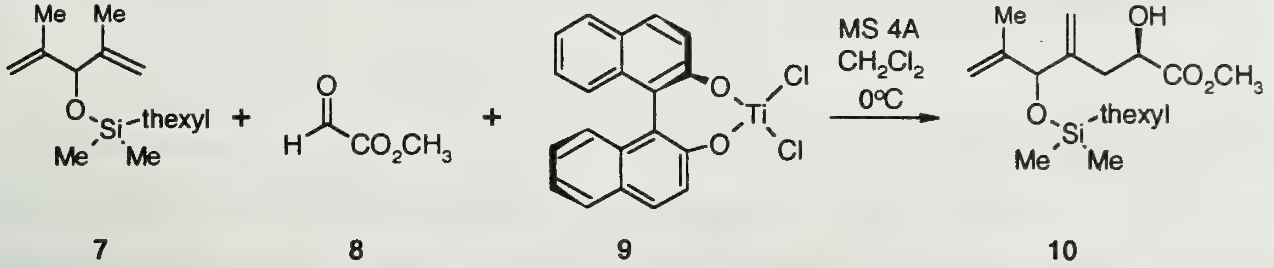


3 R = 3-MeC$_6$H$_4$ 4 5 6

(R,R)-DIOP = (R,R)-2,3-*O*-isopropylidine-2,3-dihydroxy-1,4-bis(diphenylphosphino)butane

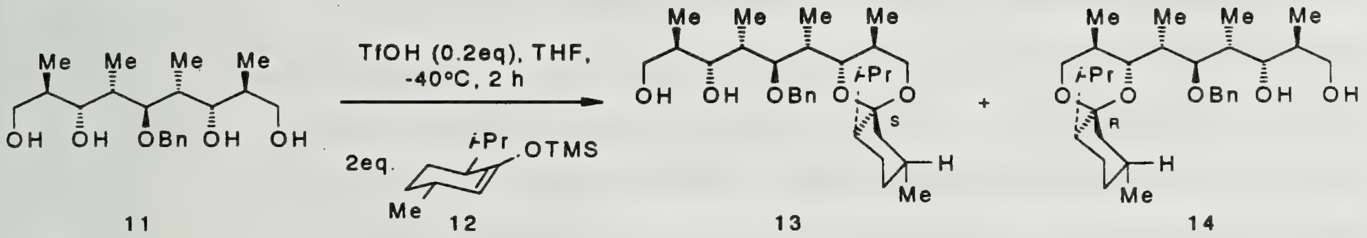
A catalytic carbonyl-ene reaction has been introduced that results in asymmetric C-C bond formation.[7] Bis-allylic silyl ether **7** reacted with the prochiral glycolate **8** in the presence of the chiral titanium complex **9** to yield **10** with >99% syn diastereoselectivity and 99% ee, Scheme 4. Catalyst **9** was prepared from enantiomerically pure binaphthol. This reaction is one of the few end termini reactions that result in carbon-carbon bond formation.

Scheme 4



Divinyl carbinols are not the only example of substrates capable of undergoing terminus selective reactions of achiral and meso chains. Terminus differentiation has also been achieved for achiral polyols and achiral acids. Menthone derivatives have been used to differentiate enantiotopic termini in various polyols with success. One example employing menthone as the terminus selective reagent is found in the synthesis of the C(19)-C(27) segment of Rifamycin S, Scheme 5.[3c] Meso tetrol **11** was treated with *d*-menthone TMS enol ether **12** in the presence of triflic acid for two hours to yield the menthonides **13** and **14** in a 4.5/1 ratio in favor of the desired menthonide **13**. In this example, the problem of enantiotopic terminus differentiation was reduced to separation of diastereomers. Other examples of the use of menthonides are 1,3-diols,[8] meso diols,[9] and pentane triols.[10]

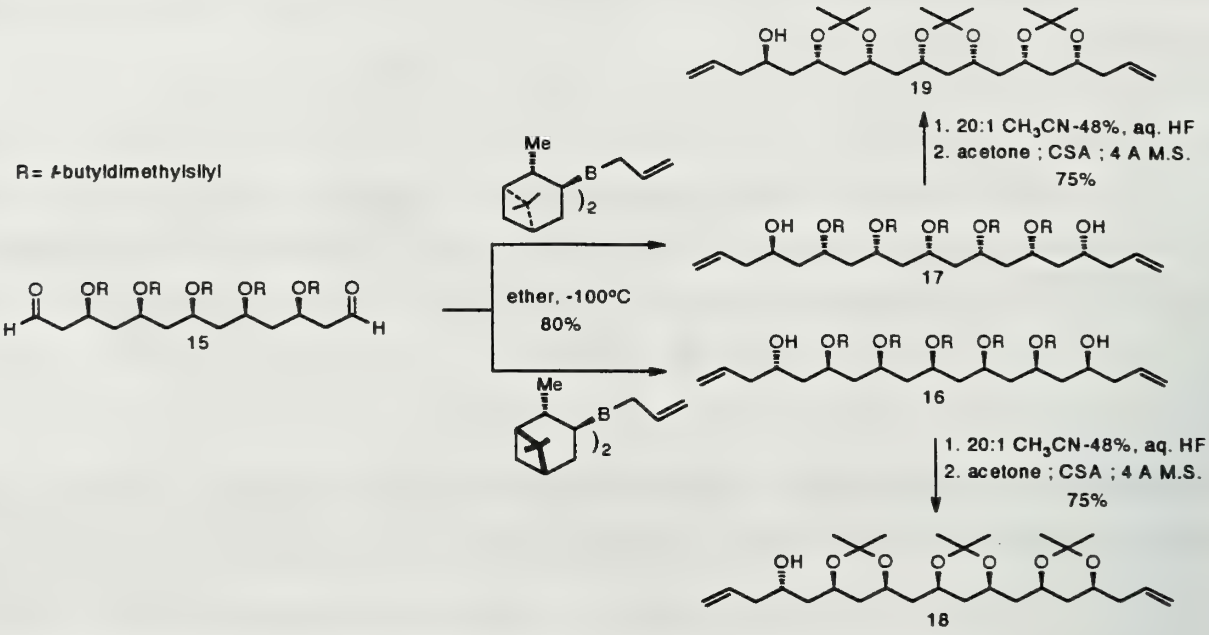
Scheme 5



An interesting example of terminus differentiation using diisopinylcampheyl(allyl)borane to give either antipode of a meso chain is shown in Scheme 6.[3b] Dialdehyde **15** reacted with either (+)- or (-)-diisopinylcampheyl(allyl)borane to yield either **16** or **17** with >15/1 diastereoselectivity and > 98% ee. After deprotection, acetonide formation resulted in the tris(acetonides) **18** or **19**. A 15/1 selectivity was observed in favor of the thermodynamically favored syn 1,3-acetonide. The selectivity is explained by the

1,3-diaxial interactions that are present with the minor product. Other terminus selective reactions for achiral and meso chains have been developed.[11]

Scheme 6

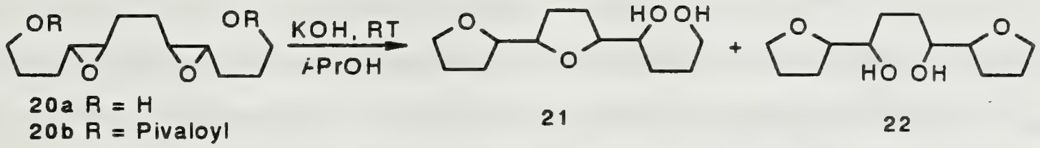


C_2 **Symmetric Chains.** Synthesis of enantiomerically pure C_2 symmetric linear molecules proceeds by one of the following methods. If the target contains a prostereogenic center (achiral), elongation occurs by the double addition of enantiomerically pure reagents to both termini. Later reactions can involve substrate and/or reagent control to add more stereogenic centers.[1] Elongation of chiral targets proceed by substrate and/or reagent control. Many syntheses have been completed that contain C_2 symmetric chains.[12]

There are two techniques used to differentiate the homotopic termini of C_2 symmetric chains. One commonly employed approach is monofunctionalization. Monofunctionalization involves the addition of a reagent, such as a protecting group, to the termini of the chain resulting in a statistical ratio of products. The maximum theoretical yield is 50% and the double addition products must be separated. Yields can be increased by recovering and recycling unreacted starting material. One example is the synthesis of mycoticin A.[13]

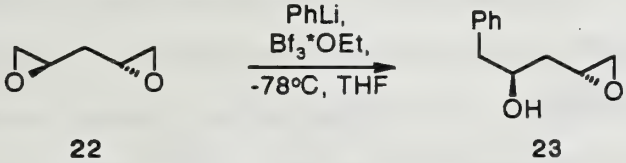
A very different approach to monofunctionalization has been developed that involves modification of the rates of removal of a protecting group and the following intramolecular functionalization (Scheme 7).[14] In this example different conditions were tested to alter the rate of deprotection of various protecting groups. Reaction of **20a** in a basic solution affords **21** and **22** in a 1.2/1 ratio. By protecting both alcohols as the pivalate esters and carrying out the reaction in isopropyl alcohol, the product ratio changed to 49/1 in favor of the desired product **21**. The observed selectivity is believed to be a result of the deprotection and cyclization rates. Under these conditions, deprotection is believed to be slower than cyclization.

Scheme 7



Steric proximity effects have also proved useful in monofunctionalization reactions. One such example is the boron-trifluoride-activated, epoxide opening with phenyllithium, Scheme 8.[15] Complexation of the epoxide oxygen with boron increases the rate of epoxide opening. The authors hypothesized that the sterics effects introduced by the first phenyl addition inhibit activation of the other epoxide decreasing the rate for the second addition. Enantiopure diepoxide **22**, synthesized from 2,4-pentanedione, reacted with phenyllithium in the presence of boron trifluoride to yield monoepoxide **23** in 79% yield and >97% enantiomeric excess.

Scheme 8

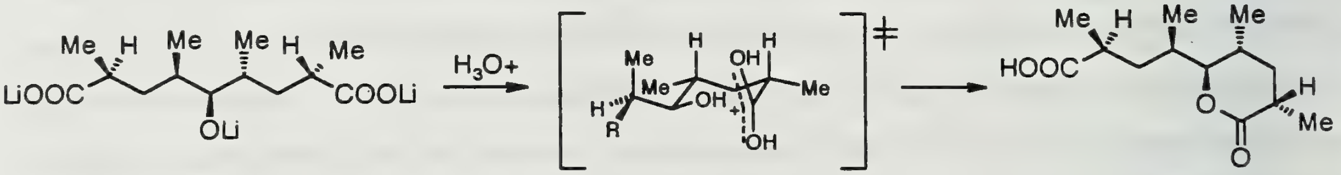


Pseudo-C_2 Symmetric Chains. Pseudo-C_2 symmetric chains are characterized by the existence of a central chirotopic, nonstereogenic carbon.[1] Pseudo-C_2 symmetric chains can be synthesized by the addition of an enantiomerically pure reagent to the termini of an achiral chain. Reagent control strategies are then used to introduce new stereogenic centers.[4] Pseudo-C_2 symmetric chain synthesis has been applied to the synthesis of peptides,[16] synthesis of the C(10)-C(18) fragment of FK 506,[17] and the ansa chain of streptovaricin A.[18]

Desymmetrization of pseudo-C_2 symmetric chains requires the use of diastereotopic group selective reactions. Differentiation requires the conversion of the central nonstereogenic carbon into a stereogenic atom.[1] Examples of pseudo-C_2 symmetric reactions are the least common because relatively few fragments of interest contain this symmetry.[4]

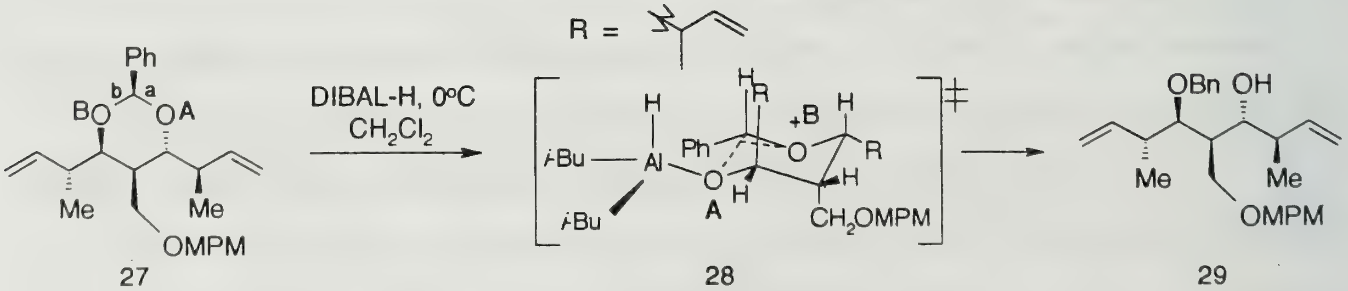
Often selectivity is governed by the formation of chair-like transition state that places some substituents in an unfavorable environment. The kinetic lactonization of 2,4,6,8-tetramethyl-5-hydroxyazelaic acids is an example of the process (Scheme 9, **24**).[19] In this acid-catalyzed reaction, the two termini were found to undergo lactonization at significantly different rates. A discussion of the ground state conformations was provided to explain the >350/1 selectivity in favor of lactone **26**. Preclosure conformation **25** was found to be the only likely transition state, all other possibilities either contained 1,3-diaxial interactions or the positioning of a very large group in the axial position. Kinetic lactonization is a very efficient means of terminus differentiation and has also been used by in the synthesis of FK 506.[17] Iodolactonization has been used to differentiate olefins of a heptadienoate with a 147/1 olefin selectivity.[20]

Scheme 9



Diisobutylaluminum hydride reduction of benzylidene acetals has also been employed for the desymmetrization of a pseudo-C_2 symmetric chain.[18] Benzylidene acetal **27** (Scheme 10) was selectively converted into the benzyl ether **29** in 89% yield with >20/1 selectivity. According to the X-ray crystal structure, both the phenyl and one of the *sec*-butenyl groups are equatorial whereas the other *sec*-butenyl and the (phenyl, (4-methoxy)benzyloxymethyl) groups are in axial positions. Transition structure **28** was offered to explain the selectivity in forming **29**. An X-ray analysis of **27** revealed that bond **a** and bond **b** are 1.417 Å and 1.404 Å respectively. Anomeric lengthening of bond **a** leads to a decreased 1,3-diaxial interaction and occupies an electron pair of the oxygen at bond **b**. Therefore complexation of diisobutylaluminum hydride only occurs with an electron pair of oxygen **A**.[21]

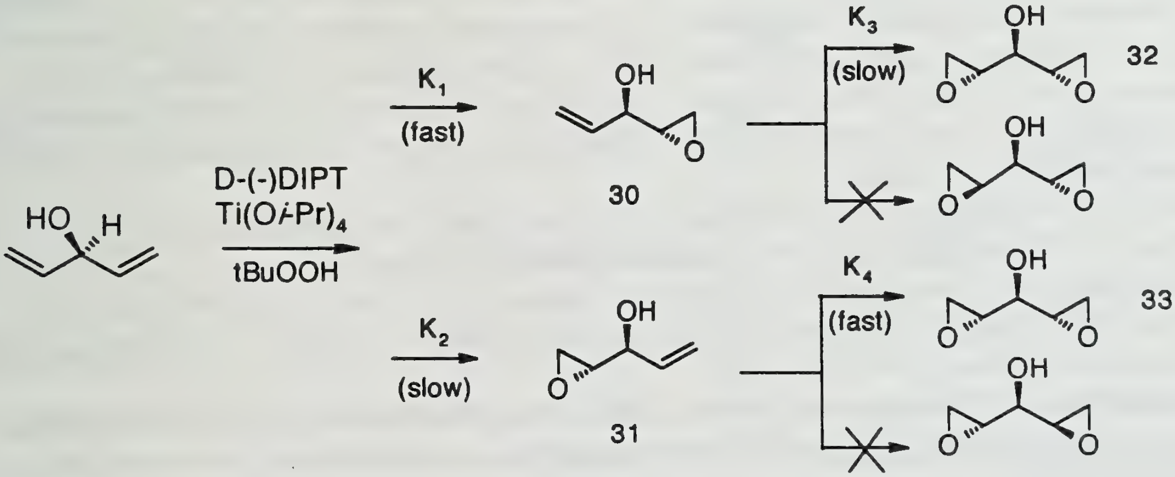
Scheme 10



ENANTIOMERIC EXCESS ENHANCEMENT VIA KINETIC RESOLUTION

Many reactions previously discussed have displayed an enhancement of enantiomeric excess as a function of time. For the Sharpless asymmetric epoxidation (Scheme 11) the enantiomeric excess was observed to increase from 84% at 3 hours to >97% after 140 hours.[2] Utilization of (-)-DIPT led to an increased rate of formation of **30** (K_1/K_2 = 104) relative to **31**. Epoxide **31** is consumed to give epoxide **33** as soon as it is formed because K_4 is large relative to K_2 (K_4/K_2 = 104) but epoxide **30** remains (K_3 is slow). The double addition products (**32** and **33**) are easily separated to leave enantiopure **30**. Enantiopure products are attainable by this process if the following criteria are met: (1) the kinetics of the first addition must strongly favor one product, (2) a second addition must be possible; (3) the first addition must not affect the rate of the second addition, (4) the reaction must go to completion. If the difference in the rate of the first addition is small, enantiopure products may be obtainable at the expense of the yield. Reaction yield will be inversely related to enantiomeric purity because double addition products are discarded (substrate is consumed in forming the double addition products). Enhancement of enantiomeric excess in an asymmetric synthesis via kinetic resolution is a very desirable feature of two directional chain synthesis.

Scheme 11



CONCLUSION

Two directional chain synthesis has already been successfully employed in complex syntheses to give products with extraordinarily high levels of enantiomeric purity. If group- and face-selective reactions are used, kinetic resolution can be applied to give enantiopure products. Two directional chain synthesis is dependent upon efficient terminus selective transformations. Examples of terminus selective transformations for homotopic, enantiotopic, and diastereotopic termini have been presented. Although many examples of terminus selective reactions exist, there is still need for development. Two directional chain synthesis is a very efficient technique that can be employed to synthesize fragments used to construct many complex natural products.

References

(1) Poss, C.S.; Schreiber, S.L. *Acc. Chem. Res.* **1994**, 27, 9.

(2) Schreiber, S.L.; Schreiber, T.; Smith, D. *J. Am. Chem. Soc.* **1987**, 109, 1525.

(3) (a) Burke, S.D.; Buchanan, J.L.; Rovin, J.D. *Tetrahedron Lett.* **1991**, 32, 3961. (b) Wang, Z.; Deschenes, D. *J. Am. Chem. Soc.* **1992**, 114, 1090. (c) Harada, T.; Kagamihara, Y.; Tanaka, S.; Sakamoto, K.; Oku, A. *J. Org. Chem.* **1992**, 57, 1637. (d) Schreiber, S.L.; Goulet, M.T. *J. Am. Chem. Soc.* **1987**, 109, 4718. (e) Schreiber, S.L.; Goulet, M.T. *J. Am. Chem. Soc.* **1987**, 109, 8120.

(4) Magnuson, S. *Tetrahedron*, **1995**, 51, 2167.

(5) Kobayashi, Y.; Kato, N.; Shimazaki, T.; Sato, F. *Tetrahedron Lett.* **1988**, 29, 6297.

(6) (a) Tamao, K.; Tohma, T.; Inui, N.; Nakayama, O.; Ito, Y. *Tetrahedron Lett.* **1990**, 31, 7333.

(7) Mikami, K.; Narisawa, S.; Shimizu, M.; Terada, M. *J. Am. Chem. Soc.* **1992**, 114, 6566.

(8) Harada, T.; Hayashiya, T.; Wada, I.; Iwa-ake, N.; Oku, A. *J. Am. Chem. Soc.* **1987**, 109, 527.

(9) Harada, T.; Ikemura, Y.; Nakajima, H.; Ohnishi,, T.; Oku, A. *Chem. Lett.* **1990**, 1441.

(10) Harada, T.; Wada, I.; Uchimura, J.; Inoue, A.; Tanaka, S.; Oku, A. *Tetrahedron Lett.* **1991**, 32, 1219.

(11) Fuji, K.; Node, M.; Terada, S.; Murata, M.; Nagasawa, H. *J. Am. Chem. Soc.* **1985**, 107, 6404.

(12) (a) Marshall, J.A.; Beaudoin, S.; Lewinski, K. *J. Org. Chem.* **1993**, 58, 5876. (b) Poss, C. S.; Rychnovsky, S.D.; Schreiber, S.L. *J. Am. Chem. Soc.* **1993**, 115, 3360. (c) Saito, S.; Hamano, S.; Moriyama, H.; Okada, K.; Moriwake, T. *Tetrahedron Lett.* **1988**, 29, 1157. (d) Nakahara, Y.; Fujita, A.; Ogawa, T. *Agric. Biol. Chem.* **1987**, 51, 1009. (e) Hoye, T.R.; Hanson, P.R.; Kovelesky, A.C.; Ocain, T.D.; Zhuang, Z. *J. Am. Chem. Soc.* **1991**, 113, 9369. (f) Ikemoto, N.; Schreiber, S.L. *J. Am. Chem. Soc.* **1990**, 112, 9657. (g) Ikemoto, N.; Schreiber, S.L. *J. Am. Chem. Soc.* **1992**, 114, 2524.

(13) Poss, C.S.; Rychnovsky, S.D.; Schreiber, S.L. *J. Am. Chem. Soc.* **1993**, 115, 3360.

(14) Hoye, T.R.; Witowski, N.E. *J. Am. Chem. Soc.* **1992**, 114, 7291.

(15) Rychnovsky, S.D.; Griesgraber, G.; Zeller, S.; Skalitzky, D.J. *J. Org. Chem.* **1991**, 56, 5161.

(16) Babine, R.E.; Zhang, N.; Jurgens, A.R.; Schow, S.R.; Desai, P.R. James, J.C.; Semmelhach, M.F. *Bioorg. Med. Chem. Lett.* **1992**, 2, 541.

(17) (a) Schreiber. S.L.; Sammakia, T.; Uehling, D.E. *J. Org. Chem.* **1989**, 54, 15. (b) Nakatsuka, M.; Ragan, J.A.; Sammakia, T.; Smith, D.B.; Uehling, D.E.; Schreiber, S.L. *J. Am. Chem. Soc.* **1990**, 112, 5583.

(18) (a) Schreiber, S.L.; Wang, Z.; Schulte, G. *Tetrahedron Lett.* **1988**, 29, 4085. (b) Wang, Z.; Schreiber, S.L. *Tetrahedron Lett.* **1990**, 31, 31.

(19) Hoye, T.R.; Peck, D.R.; Swanson, T.A. *J. Am. Chem. Soc.* **1984**, 106, 2738.

(20) Kurth, M.J.; Brown, E.G.; *J. Am. Chem. Soc.* **1987**, 109, 6844.

(21) Denmark, S.E.; Almstead, N.G. *J. Am. Chem. Soc.* **1991**, 113, 8089.

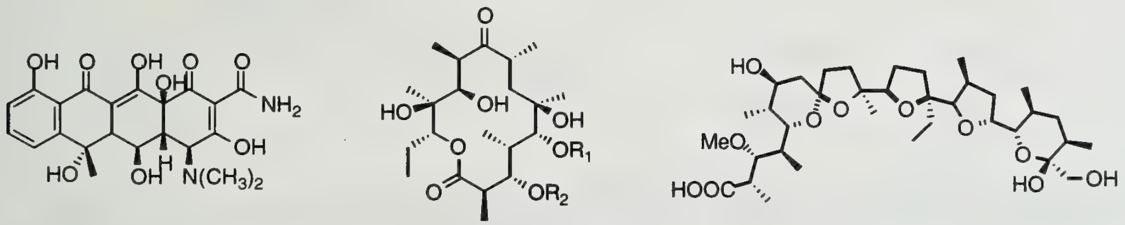
MACROLIDE POLYKETIDE BIOSYNTHESIS AND GENETICALLY ENGINEERED NOVEL POLYKETIDE METABOLITES

Reported by Deborah S. Mortensen February 10, 1997

INTRODUCTION

Polyketides form a diverse class of natural products and have been the focus of both synthetic and biosynthetic investigations due to their structural diversity and potential antibiotic, immunosuppressant, antitumor, or other pharmacological activity.[1] Examples of important polyketides include the antibiotics oxytetracycline (**1**), erythromycin A (**2**), and monensin A (**3**), aromatic, macrolide, and polyether polyketides respectively (Figure 1). Polyketide synthases (PKSs) are the enzymes responsible for the biosynthesis of the polyketides. PKSs are classified into sub-types. Type I PKSs are multifunctional proteins responsible for the synthesis of macrolides and polyethers. Type II PKSs are comprised of a single set of monofunctional proteins and catalyze the synthesis of the aromatic polyketides. The nature of the PKSs have made them useful in the genetic engineering of novel polyketides by mutation and recombinant DNA strategies. The genetic manipulation of natural product biosynthesis can lend new insight to the function and mechanism of the enzymes involved. Additionally, random or selective mutations may yield a whole series of novel molecules or specifically tailored potential drugs. This report will cover type I PKSs and novel polyketide metabolites produced through their genetic manipulation.

Figure 1



Oxytetracycline
(**1**)

Erythromycin A (**2**)
(R_1=desoamine, R_2=cladinose)

Monensin A
(**3**)

BIOSYNTHESIS

The general mechanism for polyketide biosynthesis is outlined in Figure 2.[1] Polyketides are biosynthesized in successive decarboxylative condensations of acyl units, similar to fatty acid synthesis.[1-3] In step 1 a chain extender unit is loaded onto an acyl carrier protein (ACP) from coenzyme A by an acyl transferase (AT). Step two is a stereospecific decarboxylative condensation with a starter unit, bound to a ß-keto synthase (KS) domain via a thioester linkage. In fatty acid synthesis, the condensations are generally followed by the complete reduction of ß-keto group to a methylene, completing steps 3-5. In polyketide synthesis, however, the degree of reduction in each round of chain extension may vary. The ß-keto thioester produced in step two may be reduced by ß-keto reductase (KR) to the hydroxyl in step 3 or

Copyright © 1997 by Deborah S. Mortensen

be transferred to a KS to enter another chain cycle, skipping straight to step 6. Elimination by a dehydratase (DH) to an olefin in step 4 can follow the KR or again the chain can enter a new cycle. In step 5 the partially reduced product may be reduced completely to a methylene by an enoyl reductase (ER). The resulting chain is then transferred to a KS, step 6, for another chain extension cycle. After the completion of the final cycle, a thioesterase (TE) domain catalyzes the release of the polyketide chain from the enzyme complex.

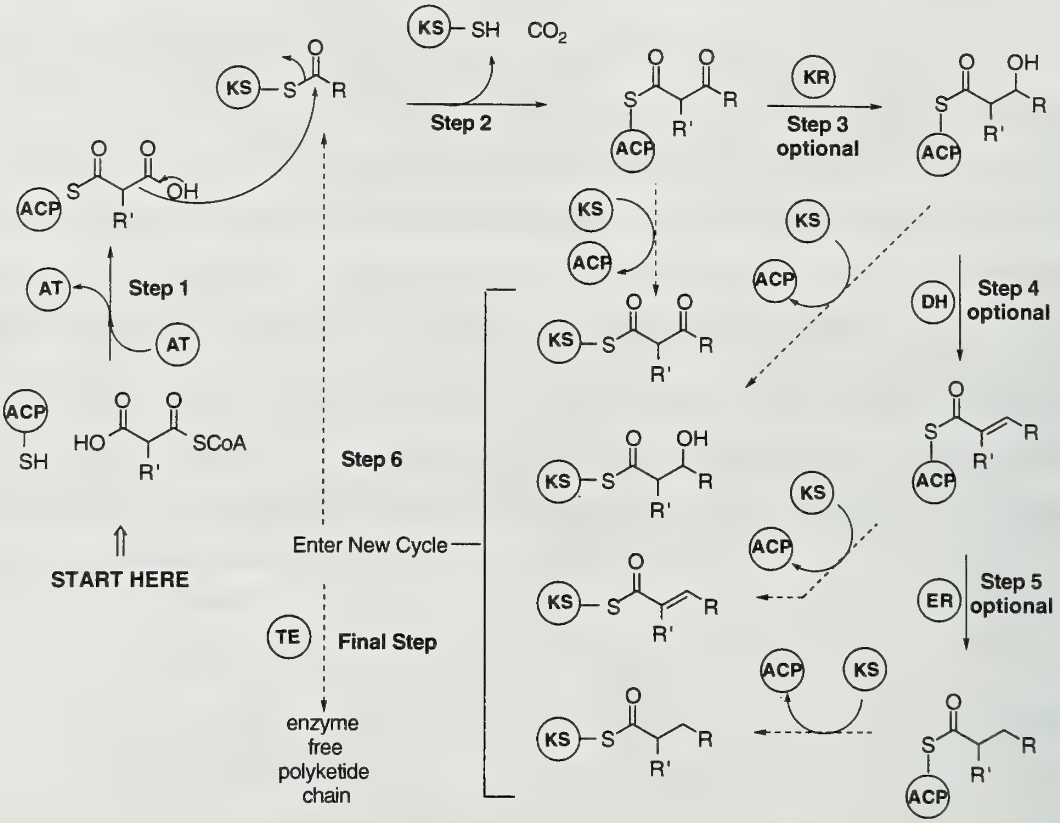


Figure 2. Proposed mechanism for a cycle of polyketide biosynthesis.

Polyketide synthases achieve the vast structural diversity of their products through variation in selection of starter unit, usually acetate, propionate, or butyrate, chain length determination (number of cycles), stereochemistry and degree of reduction of the ß-keto group in each chain extension cycle. Post polyketide modifications, such as oxidations, reductions, alkylation or glycosylations may convert the polyketide product into a potentially bioactive compound.[4]

MODULAR POLYKETIDE SYNTHASES

Type I PKSs catalyze the production of the macrolide antibiotics. Genetic analysis,[5,6] site directed mutagenesis,[5,7] and feeding experiments[8] have helped to formulate a model for these modular PKSs. The model for type I PKSs is exemplified by the gene cluster and proteins coding for 6-deoxyerythronolide-B (6-deB) **(4)**, a precursor to erythromycin **(2)** (Figure 3). The gene cluster has three open reading frames (ORFs), with each ORF coding for a large multifunctional protein (DEBS1-3).[5]

Each ORF can be further broken down into two modules, with each module containing all of the active sites necessary for one round of polyketide synthesis. The choice of extender unit, stereochemistry of condensation and degree of reduction for one cycle is determined by domains present in each module. There are six modules with a total of 28 active sites and there appears to be an unique active site for every step in the biosynthesis.[5] Further investigations have determined that not only is there a site for every step, but remarkably the genetic order reflects the biochemical order of events.[5,7,9-11] For example, module 3 does not contain any active reductive domains and results in the retention of keto group at C9. Module 4 contains all reductive domains, KR, DH, and ER, resulting in complete reduction to a methylene at C7. Thus, it is possible to predict the structure of the polyketide from the gene sequence coding for the PKS.

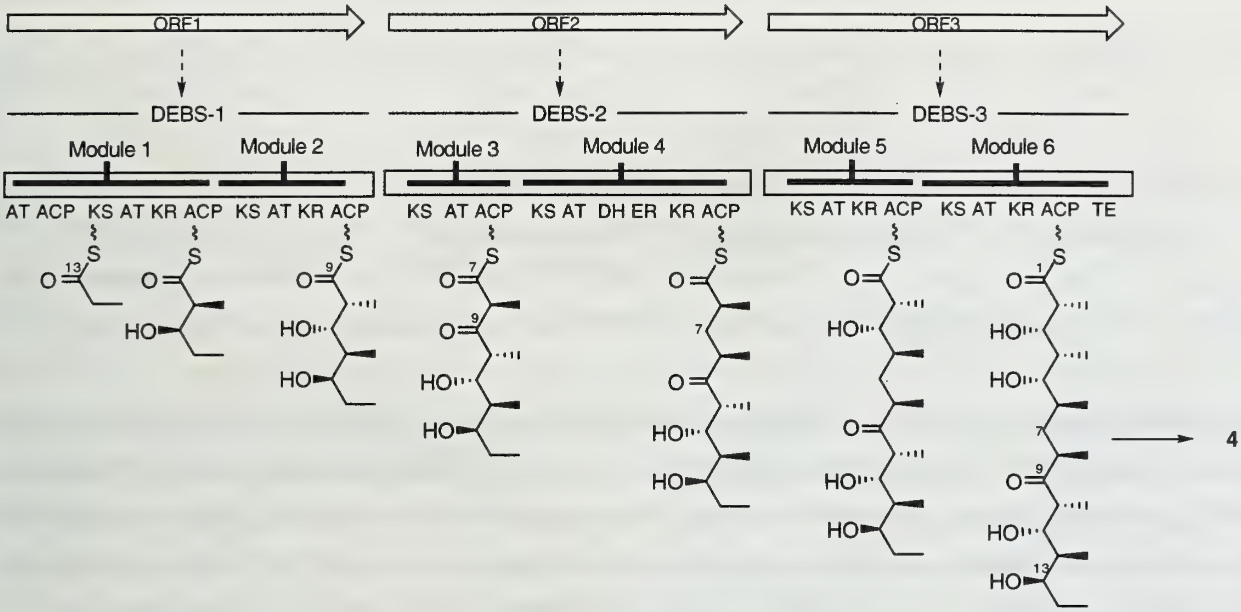
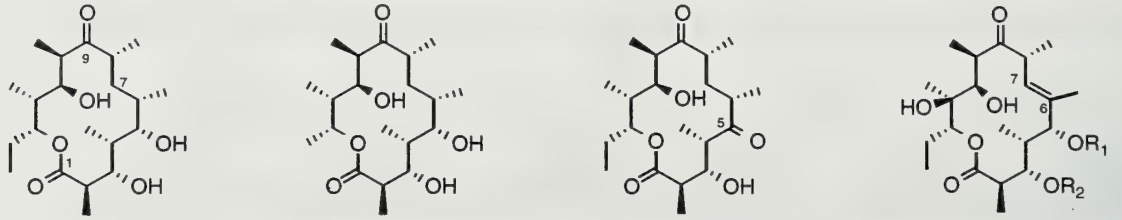


Figure 3. The modular 6-deoxyerythronolide-B synthase (DEBS) and the protein bound intermediates in the biosynthesis of 6-deoxyerythronolide-B (6-deB).

The gene cluster coding for DEBS1-3 proteins spans a 32 kb region. The large size of the PKS gene cluster makes its manipulation on plasmids difficult.[12] Khosla and coworkers reported the development of an in vivo recombination technique to clone these large genes into a plasmid for expression in a heterologous host.[12] The expression plasmid was constructed by the recombination of temperature-sensitive shuttle plasmids and recipient plasmids, using techniques described by Bender et al. for the construction of large DNA segments in *Escherichia coli.*[13] The plasmid is then recovered from the *E. coli* and transferred into a host species for expression. The large DEBS proteins were successfully expressed and produced 6-deB (**4**) and 8,8a-deoxyoleandolide (**5**), a lactone identical to 6-deB except for a methyl group side chain in place of a normal ethyl group (Figure 4). This second product arises from the selection of acetate as a starter unit in place of propionate. These recombinant techniques have been used to express both wild-type and hybrid PKSs.

NOVEL MACROLIDE POLYKETIDES

Novel derivatives of the macrolide 6-deB **(4)** have been prepared by site directed mutagenesis in the gene cluster coding for DEBS. A selective mutation that disabled the keto reductase element in module 5 resulted in the formation of 5,6-dideoxy-5-oxoerythronolide B **(6)**, which bears a keto group at C5 as opposed to the hydroxy group in 6-deB.[5] Similarly, the disabling of the enoyl reductase domain of module 4 resulted in the *in vivo* production of $\Delta^{6,7}$-anhydroerythromycin C **(7)** (Figure 4).[7] The structure of these products supports the linear ordered action of the model as specific domain changes were shown to affect single sites in the polyketide product. They have also demonstrated the ability of the PKS to process an altered intermediate chain. Changing the action in one domain did not prevent the subsequent domains from functioning normally.



6-deB
(4)

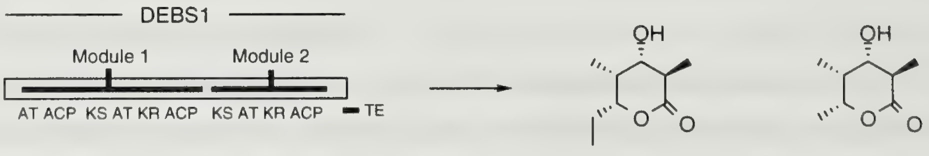
8,8a-deoxyoleandolide
(5)

5,6-dideoxy-5-oxo-erythronolide B **(6)**

$\Delta^{6,7}$-Anhydroerythromycin C **(7)**
(R_1=desoamine, R_2=cladinose)

Figure 4. 6-Deoxyerythronolide-B (6-deB) and mutants generated by site directed mutagenesis.

Macrolide ring size can be manipulated by the creation of truncated PKSs. Relocation of the genes coding for the thioesterase (TE) domain, which is responsible for chain termination and cyclization of the polyketide product, allows for the production of truncated polyketide chains. In the hybrid DEBS1-TE, the TE domain from module 6 has been relocated to the carboxyl terminus of DEBS1 through recombinant DNA technology.[14-16] The successful expression of DEBS1-TE by the hybrid gene resulted in the biosynthesis of the triketide lactones **8** and **9** (Figure 5). The lactone **9** results from the incorporation of acetate as a starter unit instead of propionate, demonstrating a relaxed starter unit specificity that was also observed for the entire PKS. The triketide lactone **8** was also produced by DEBS1 alone, but in much lower yield.[17] The increased production of **8** by DEBS1-TE suggests that the TE domain plays a role in the recognition of the terminal hydroxyl and facilitates cyclization. The ability of TE to recognize the product of DEBS1 and catalyze its release and cyclization suggests that individual domains from one module may be relocated to another without loss of activity.



8

9

Figure 5. The triketide lactones produced by hybrid DEBS1-TE.

As was demonstrated with the full DEBS proteins, site directed, genetic mutations in DEBS1-TE afforded novel metabolites.[18] The disabling of the keto reductase domain in module 2 afforded the expected keto lactones **10** and **11** (Figure 6). The keto lactones **10** and **11** were also produced by a chimeric PKS where the KR domain of module 2 of DEBS1-TE was replaced with the nonfunctional reductive domain from DEBS module 3 by recombinant assembly.[18] The experiments with DEBS1 and DEBS1-TE demonstrated the ability of the individual DEBS proteins to function independently as well as the tolerance for domain replacement.

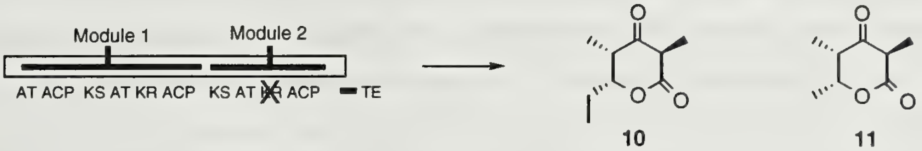


Figure 6. The keto-lactones produced by site directed mutagenesis disabling the KR domain of module 2 of DEBS1-TE.

The relaxed starter unit specificity was further explored with the development of a cell-free system for biosynthetic studies.[19-21] The DEBS1-TE enzyme was purified from cell extracts to homogeneity and retained its activity. Controlled feeding experiments demonstrated that a variety of starter units could successfully be incorporated (Figure 7). The DEBS1-TE successfully incorporated acetyl- **(12)**, *n*-butyryl- **(13)** and isobutyryl-CoA **(14)** as starter units to yield lactones **9**, **15** and **16** respectively.[21] The enzyme was shown to recognize a variety of starter units as well as correctly complete the synthesis on feedings of ketide intermediate **17**.[19,20] An unreduced metabolite **18** was produced in the absence of NADPH, demonstrating the correct processing of an unreduced intermediate by the PKS.[20] The development of the cell-free system is essential in the application of the modular PKSs to combinatorial studies. The cell-free system allows for the use of starter units, extender units, or conditions not possible *in vivo*. This expands the limitations of structural possibilities available beyond those of the *in vivo* biosynthesis.

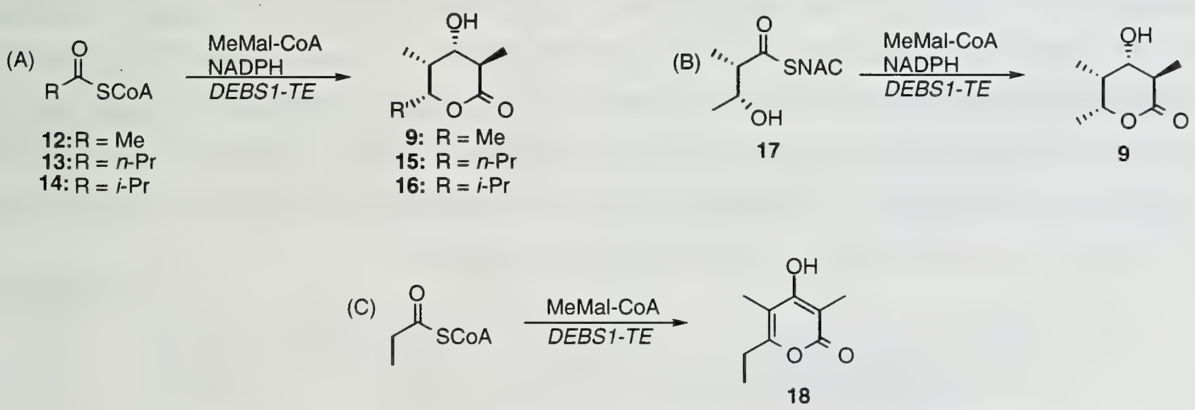


Figure 7. Novel polyketide metabolites produced in cell-free biosynthesis utilizing different starter units (A and B) and altered conditions (C).

Further experiments utilizing gene manipulation resulted in the successful production of novel polyketide lactones (Figure 8). A genetic hybrid composed of gene clusters coding for DEBS modules 1-3 with the TE domain fused to the carboxyl terminus resulted in the production of the tetraketides **19** and **20**.[17] The two products arise from the same linear polyketide chain, and **20** is hypothesized to result from the nonenzymatic decarboxylation of the free keto acid, followed by hemiketal formation. Production of **19** and **20** demonstrated that module 3 action does not depend on the association with module 4. Thus the gene cluster for an entire DEBS protein is not necessary for successful protein expression and subsequent biosynthesis; the individual modules or domains can function independently. A recombinant strain composed of DEBS modules 1-4 and the KS, AT, and KR domains of module 5 fused to the ACP and TE domains of module 6 produced metabolite **21**, one acyl unit shorter than 6deB (**4**), having completed one less cycle of chain growth.[16] The biosynthesis of **21**, utilizing the hybrid 5-6 module further supports the idea that genes coding for domains from different modules can spliced together and retain activity. This suggests the potential for creating gene clusters with specific desired sequence of domains to create a target molecule.

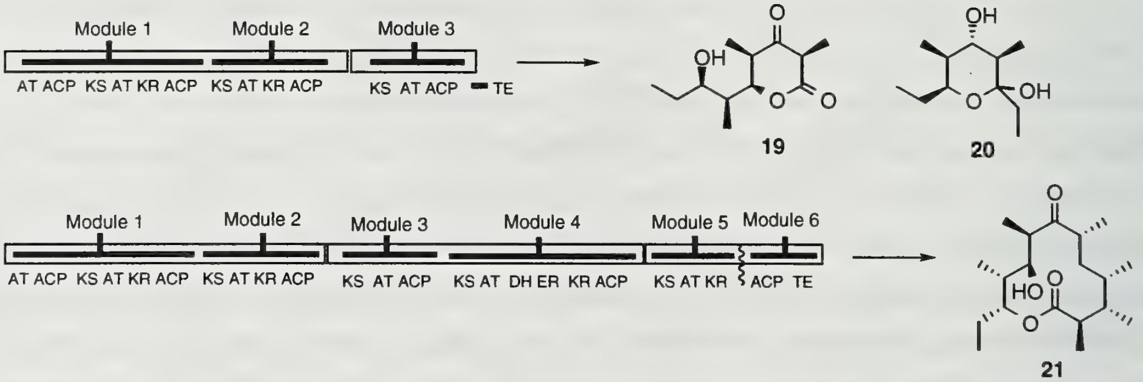


Figure 8. Tetraketides **19** and **20** produced by genetic hybrid DEBS modules 1-3+TE and hexaketide lactone **21** produced by DEBS modules1-4 with hybrid 5/6 module.

The possibility of domain swapping between PKSs coding for two different natural products was demonstrated by Leadlay et al.[22] Replacing the entire AT domain of DEBS1-TE module 1 with the AT domain from module 2 of the rapamycin producing PKS led to the biosynthetic production of two novel triketide lactones **22** and **23** (Figure 9). The structure of these lactones was expected as rapamycin is built from successive acetate condensations, while erythromycin is built from propionates. These results open up the possibility of a vast array of recombinant hybrids by domain swapping between PKSs of different strains, that could be used to produce a diverse library of compounds.

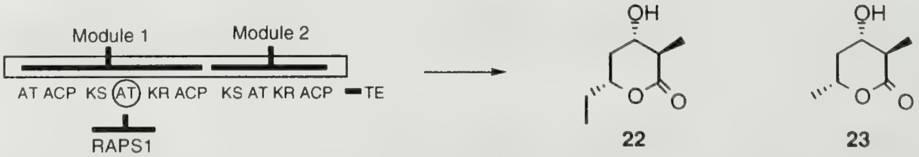


Figure 9. Novel triketide lactones produced by chimeric DEBS1-TE where AT domain in module 1 is replaced with AT from module 2 of RAPS1.

SYNTHETIC UTILITY

Genetic manipulation of natural product biosynthesis offers a method for the production of both natural products and analogous metabolites that would be less feasible to synthesize on a large scale by traditional chemical methodology. For example, 6-deoxyerythronolide-B was synthesized in a multi-step, convergent synthesis by Evans et al. (Figure 10).[23,24] Twenty-six chemical reactions as well as many purifications were required to achieve a modest yield of **4**. The same starting material **25** was used in the two convergent pathways and while the stereoselectivity of the synthesis is outstanding, the same product is reached by expression of DEBS and subsequent 6-deB production. Additionally, the plasmid construct may be used multiple times and, as was previously discussed, analogous metabolites are also attainable without creating a new synthetic approach.

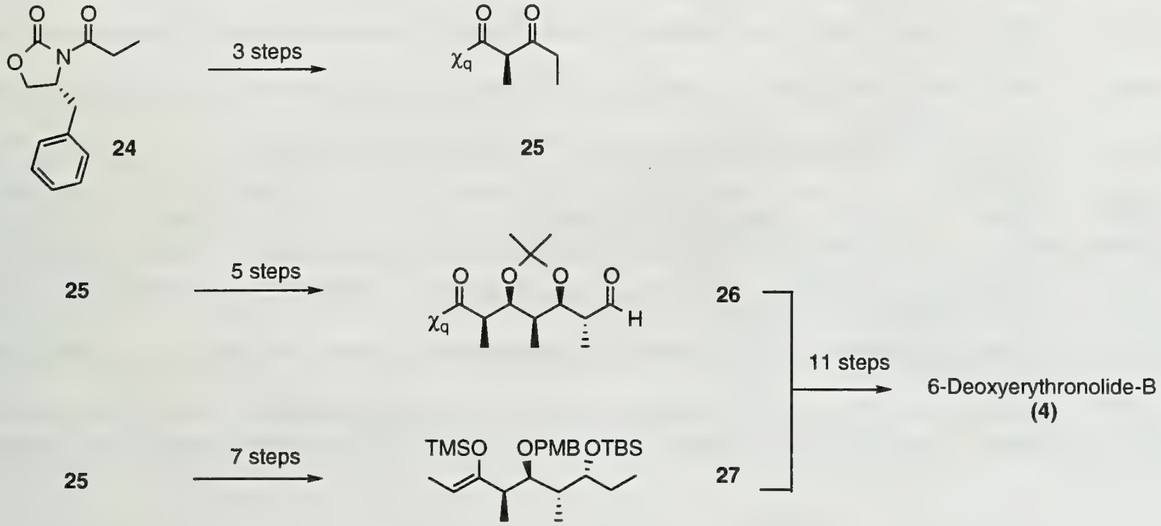


Figure 10. Outline of Evans' convergent synthesis of 6-deoxyerythronolide-B.

CONCLUSION

The results from genetic manipulation of Type I PKSs have demonstrated the great potential of these systems for generation of novel compounds by genetic engineering. The development of successful cell-free growth system allows for even greater diversity with the potential use of starter units, extender units, or conditions not possible in *in vivo* biosynthesis. The possibility of domain swapping between different PKSs expands the potential even further. As the genes encoding for other PKSs are elucidated the possible genetic combinations and subsequent novel metabolites becomes enormous.

REFERENCES

(1) O'Hagan, D. *The Polyketide Metabolites*; Ellis Horwood: New York, **1991**.
(2) O'Hagan, D. *Nat. Prod. Rep.* **1995**, *12*, 1-32.
(3) Summers, R. G.; Ali, A.; Shen, B. *Biochemistry* **1995**, 9389-402.
(4) Rohr, J. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 881-885.
(5) Donadio, S.; Staver, M. J.; McAlpine, J. B.; Swanson, S. J.; Katz, L. *Science* **1991**, 675-9.

(6) Cortes, J.; Haydock, S. F.; Roberts, G. A.; Bevitt, D. J.; Leadlay, P. F. *Nature* **1990**, *348*, 176-178.

(7) Donadio, S.; McAlpine, J. B.; Sheldon, P. J.; Jackson, M.; Katz, L. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 7119-7123.

(8) Cane, D. E.; Yang, C.-C. *J. Am. Chem. Soc.* **1987**, *109*, 1255-1257.

(9) Aparicio, J. F.; Caffrey, P.; Marsden, A.; Staunton, J.; Leadlay, P. F. *J. Biol. Chem.* **1994**, *269*, 8524-8528.

(10) Bevitt, D. J.; Cortes, J.; Haydock, S. F.; Leadlay, P. F. *Eur. J. Biochem.* **1992**, *204*, 39-49.

(11) Staunton, J. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1302-1306.

(12) Kao, C. M.; Katz, L.; Khosla, C. *Science* **1994**, *265*, 509-512.

(13) O'Connor, M.; Peifer, M.; Bender, W. *Science* **1989**, *244*, 1307-1312.

(14) Brown, M.; Cortes, J.; Cutter, A. L.; Leadlay, P. F.; Staunton, J. *J. Chem. Soc., Chem. Commun.* **1995**.

(15) Cortes, J.; Wiesmann, K.; Roberts, G. A.; Brown, M.; Staunton, J.; Leadlay, P. F. *Science* **1995**, *268*, 1487-1489.

(16) Kao, C. M.; Luo, G. L.; Katz, L.; Cane, D. E.; Khosla, C. *J. Am. Chem. Soc.* **1995**, *117*, 9105-9106.

(17) Kao, C. M.; Luo, G. L.; Katz, L.; Cane, D. E.; Khosla, C. *J. Am. Chem. Soc.* **1996**, *118*, 9184-9185.

(18) Bedford, D.; Jacobsen, J. R.; Luo, G. L.; Cane, D. E.; Khosla, C. *Chem. & Biol.* **1996**, *3*, 827-831.

(19) Pieper, R.; Luo, G.; Cane, D. E.; Khosla, C. *J. Am. Chem. Soc.* **1995**, 11373-4.

(20) Pieper, R.; Luo, G. L.; Cane, D. E.; Khosla, C. *Nature* **1995**, *378*, 263-266.

(21) Wiesmann, K.; Cortes, J.; Brown, M.; Cutter, A. I.; Staunton, J.; Leadlay, P. F. *Chem. & Biol.* **1995**, *2*, 583-589.

(22) Oliynyk, M.; Brown, M.; Cortes, J.; Staunton, J.; Leadlay, P. F. *Chem. & Biol.* **1996**, *3*, 833-839.

(23) Evans, D. A.; Kim, A. S. *Tetrahedron Lett.* **1997**, *38*, 53-56.

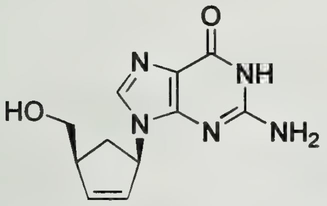
(24) Evans, D. A.; Ng, H. P.; Clark, J. S.; Rieger, D. L. *Tetrahedron* **1992**, *48*, 2127-2142.

SYNTHESES OF ANTIVIRAL CARBOCYCLIC NUCLEOSIDES

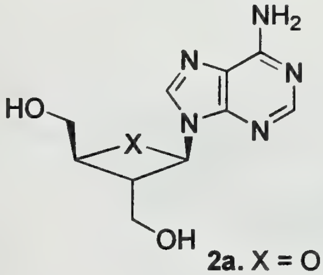
Reported by Steven Ma February 17, 1997

INTRODUCTION

The development of synthetic routes to carbocyclic nucleosides has attracted considerable attention due to the significant biological activities of these compounds as antitumor[1] and antiviral[2] drugs. Of great interest is the activity of specific derivatives as inhibitors of the human immunodeficiency virus (HIV), the agent responsible for the acquired immune deficiency syndrome (AIDS). Two carbocyclic nucleosides, (-)-carbovir[3] **(1)** and carbocyclic (-)-oxetanocin A **(2b)**,[4] have been reported to show *in vitro* activity against HIV and are currently in clinical trials. Carbocyclic nucleosides have also shown activity against herpes simplex virus (HSV) type 1 and 2, human cytomegalovirus (HCMV), and hepatitis B virus (HBV).[5] The replacement of the furanose ring oxygen with a methylene group results in greater metabolic stability of carbocyclic nucleosides against phosphorylase and hydrolase enzymes that cleave the N-glycosidic linkage of natural nucleosides.[6] This review will discuss and compare the synthetic strategies of **1** and **2b** as representative of four- and five-membered ring carbocyclic nucleosides.



1



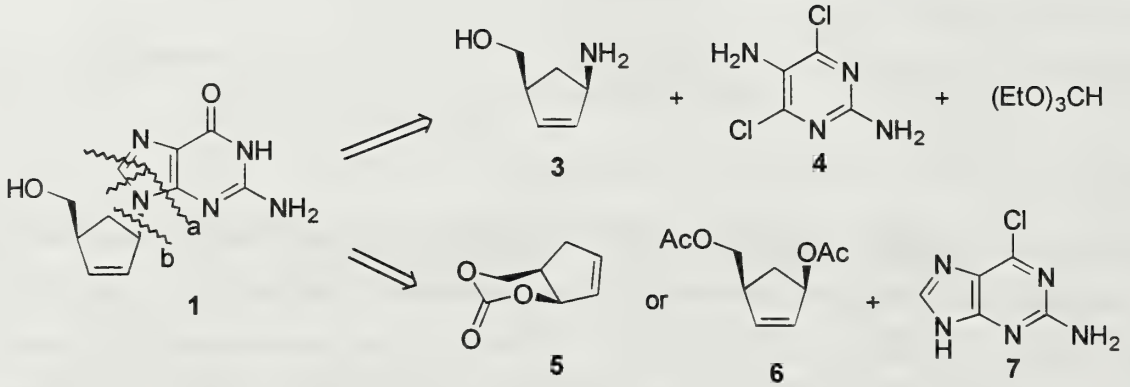
2a. X = O
2b. X = CH$_2$

RETROSYNTHETIC STRATEGIES FOR (-)-CARBOVIR

A retrosynthetic analysis of (-)-carbovir **1** shows two possible disconnections (Scheme 1). Disconnection **a** on the purine ring provides an amino alcohol (-)-**3**, 2-amino-4,6-dichloropyrimidine **4**, and triethyl orthoformate. The amino alcohol (-)-**3** has been synthesized by utilizing enzymes in resolution of a racemic lactam (±)-**9**[7] and asymmetric hydrolysis of *meso*-cyclopentene **15b**.[8] Disconnection **b** on the N-glycosidic bond suggests the palladium catalyzed coupling of allylic carbonate (-)-**5**[9] or diacetate (-)-**6**[10] with 2-amino-6-chloropurine **7**. The allylic carbonate (-)-**5** has been synthesized using vitamin B$_{12}$ and the diacetate (-)-**6** with a chiral base.

Copyright © 1997 by Steven K. Ma

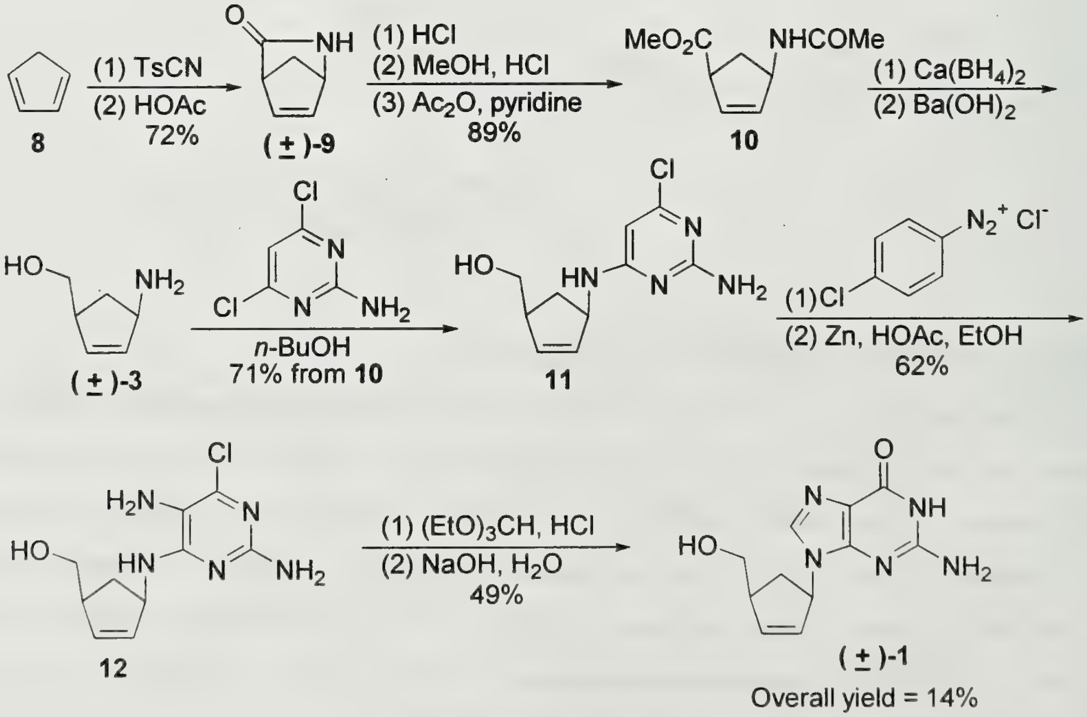
Scheme 1



FORWARD SYNTHESES OF (-)-CARBOVIR

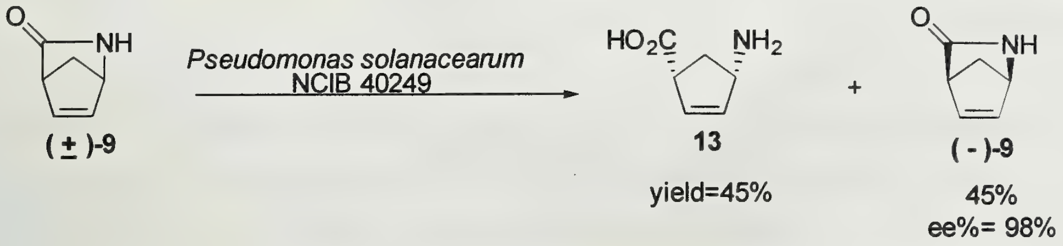
The synthesis of (±)-carbovir **1** which employed the amino alcohol (±)-**3** is outlined in scheme 2.[11] This intermediate was derived from the lactam (±)-**9**. The multi-step construction of guanine to the amino alcohol (±)-**3** was initiated by condensation of (±)-**3** with 2-amino-4,6-dichloropyrimidine **4** to yield the pyrimidinylamino derivative **11**. Diazotization of **11** with 4-chlorophenyldiazonium chloride, followed by reduction with zinc-acetic acid afforded the pyrimidine **12**. Pyrimidine **12** was converted to (±)-**1** by ring closure with triethyl orthoformate and hydrolysis with sodium hydroxide. The overall yield of (±)-**1** was 14% from cyclopentadiene.

Scheme 2



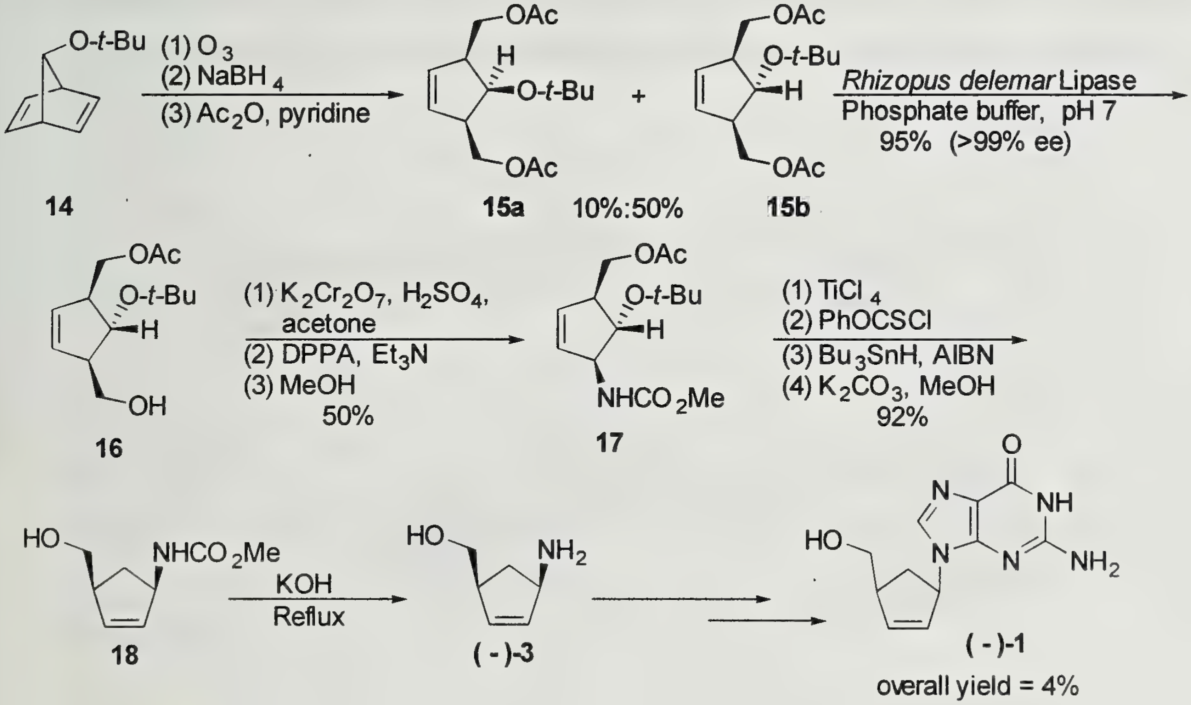
The enantiomerically enriched lactam (-)-**9** was made available by incorporating an enzymatic resolution step in the previous synthesis (Scheme 3).[7] Upon incubating lactam (±)-**9** with *Pseudomonas solanacearum* NCIB 4024, (+)-**9** was selectively hydrolyzed to the acid (+)-**13**, leaving (-)-**9** unmodified. Completion of the synthesis following Vince's[11] procedure afforded an enantiomerically enriched (-)-**1** with an overall yield of 9%.

Scheme 3



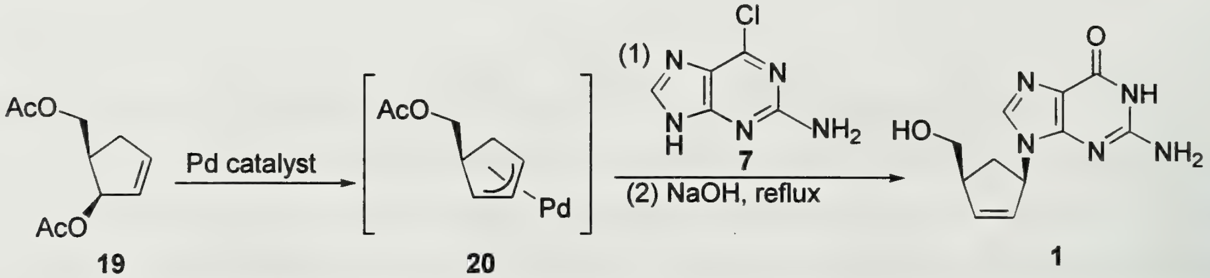
Enzymes have also been used in the asymmetric synthesis of enantiomerically pure (-)-**1**. *Rhizopus delemar* lipase catalyzed the asymmetric hydrolysis of *meso*-cyclopentene **15b** to afford the alcohol (-)-**16** with 99% enantiomeric excess and 95% yield. Hydrolysis of the ester **18** yielded the amino alcohol (-)-**3**, which was converted to (-)-**1** by following the procedure in the previous syntheses.

Scheme 4



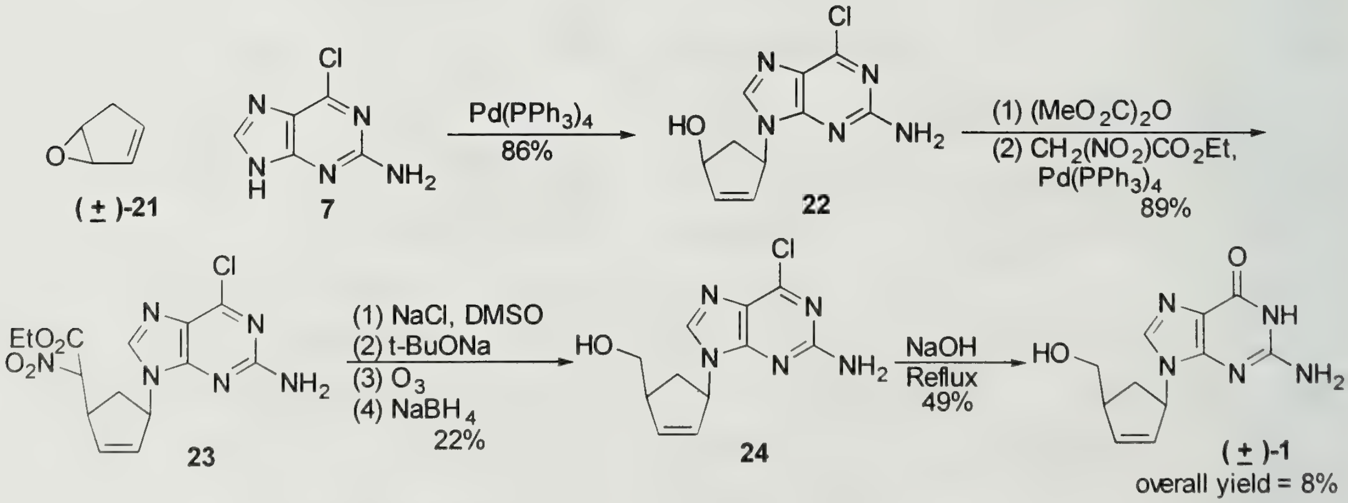
The previous syntheses of (±)- and (-)-**1** involved the multi-step construction of guanine. The use of organo-palladium chemistry to directly couple guanine precursor **7** with a functionalized cyclopentene **19** (an analog of **6**) to obtain (-)-**1** has been developed (Scheme 5).[12] This strategy is based on the chemistry of a π-allyl palladium intermediate **20**, whereupon attack by a nucleophile, such as a purine base, yielded the *cis*-1,4-disubstitued cyclopentene with the correct configuration.

Scheme 5

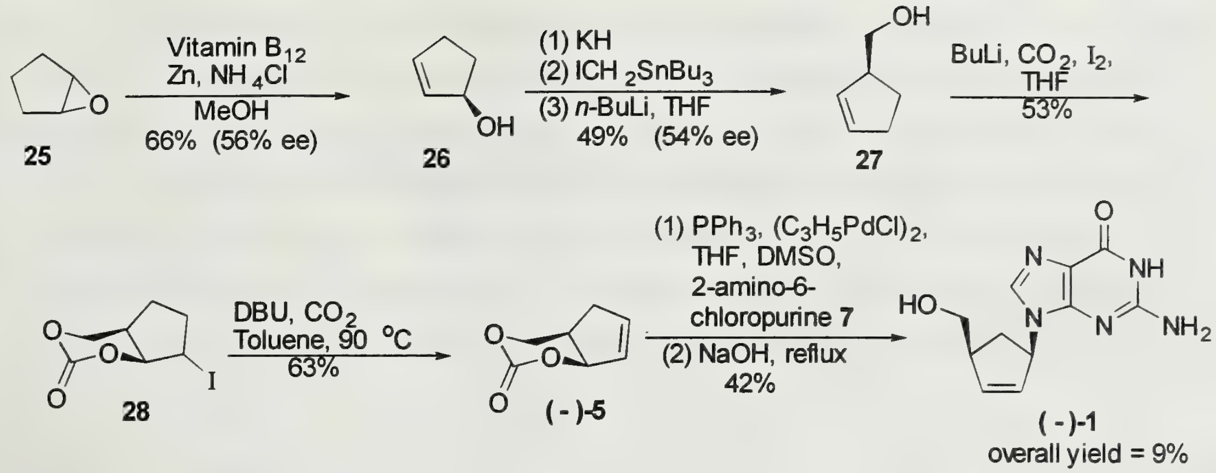


An epoxide (±)-**21** has also been used to synthesize (±)-**1** (Scheme 6).[13] Opening of epoxide (±)-**21** with 2-amino-6-chloropurine **7** under palladium(0) catalysis afforded the alcohol **22**. The disadvantage of using epoxide **21** to synthesize **1** is that the 5'-hydroxymethylene group has to be introduced after the coupling step. This requirement was eliminated by using a cyclized allylic carbonate **5** as analog of the epoxide **21** (Scheme 7).[9] The carbonate (-)-**5** was prepared by using vitamin-B_{12}-catalyzed isomerization of epoxycyclopentane **25**. Coupling of (-)-**5** with 2-amino-6-chloropurine **7** in the presence of palladium catalyst, followed by hydrolysis afforded the enantiomerically enriched (-)-**1**.

Scheme 6

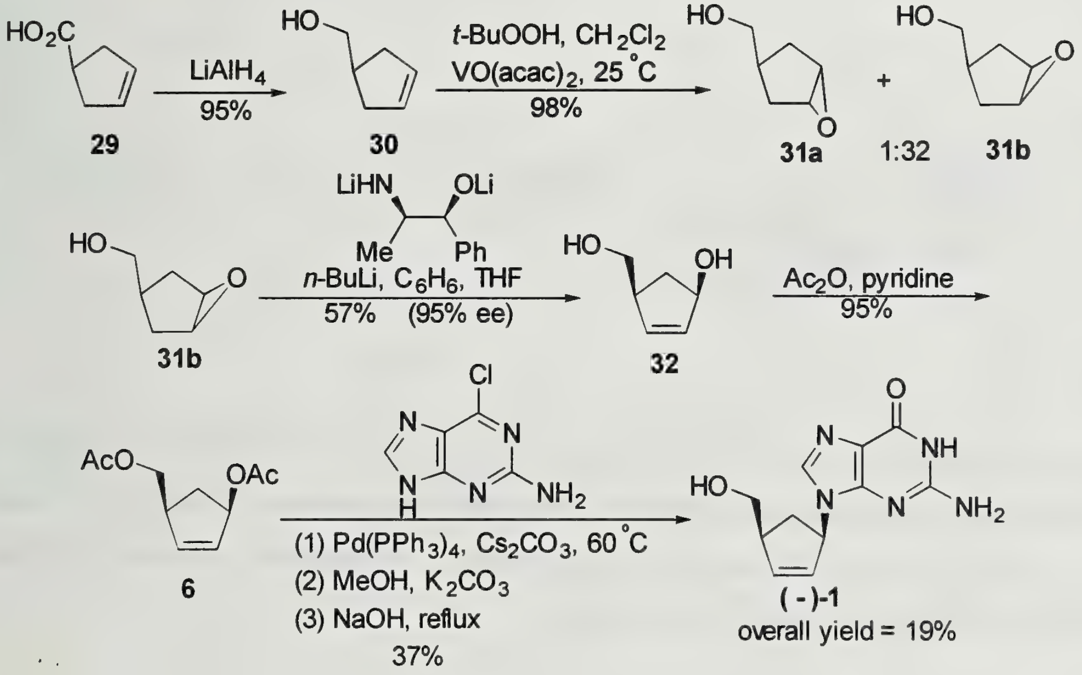


Scheme 7



An improved synthesis of (-)-**1** with the yield of 19% was developed utilizing the diacetate (-)-**6** as the intermediate.[10] The diacetater (-)-**6** was synthesized using the chiral dilithium salt of (1*S*,2*R*)-norephedrine in 57% yield with 95% enantiomeric excess (Scheme 8). The cis-epoxide **31b** rearranged upon addition of dilithiated (1*S*,2*R*)-norephedrine to yield the *cis*-diol **32**. Diacylation of the *cis*-diol **32** with acetic anhydride afforded the *cis*-diacetate (-)-**6**. Coupling of (-)-**6** with 2-amino-6-chloropurine in the presence of palladium(0) catalyst, and subsequent hydrolysis yielded enantiomerically enriched (-)-**1**.

Scheme 8

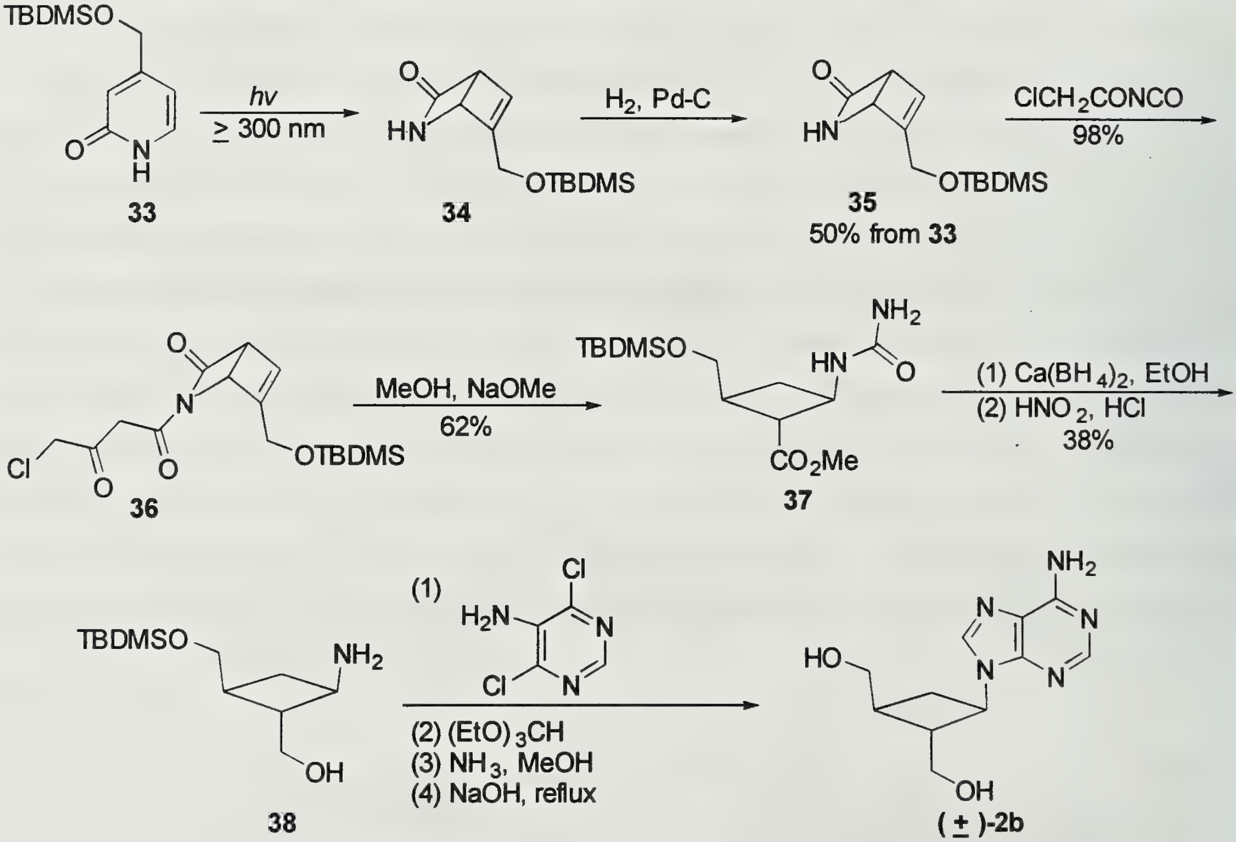


SYNTHESES OF CARBOCYCLIC (-)-OXETANOCIN A ANALOG (2b)

Another class of carbocylic nucleosides is a carbon analog of an oxetane-containing natural product. Oxetanocin A **(2a)** was isolated from *Bacillus megaterium* and is currently the only example of a naturally occurring four-membered ring nucleoside.[14] The carbocyclic oxetanocin A analog **2b** has received considerable attention because it exhibits more potent anti-HIV activity than the natural **2a**.[4]

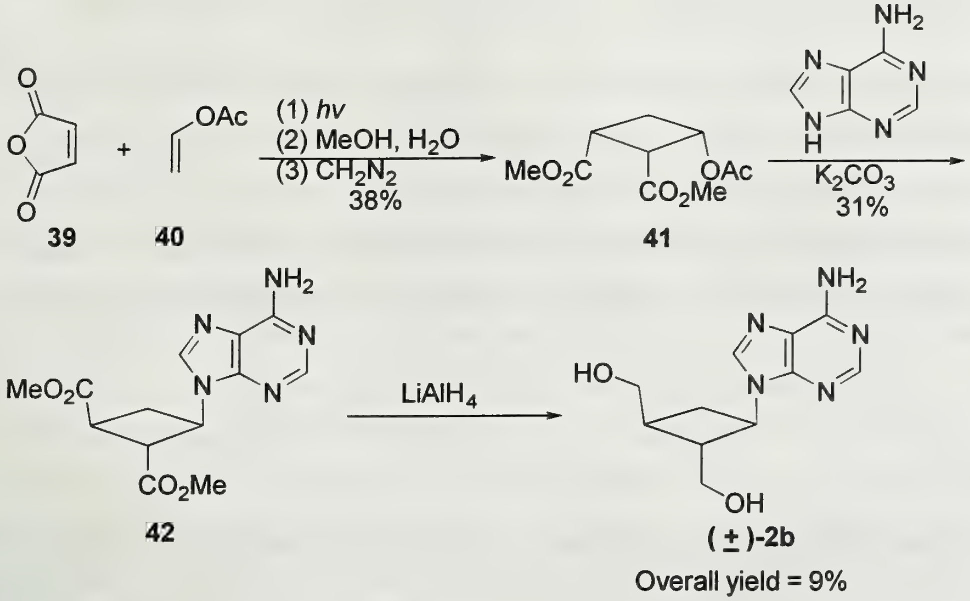
An stereoselective synthesis of **2b** was developed by utilized photochemical construction to prepare the cyclobutane intermediate (±)-**38** (Scheme 9).[15] Treatment of (±)–**38** with 2-amino-4,6-dichloropyrimidine, ring closure with triethyl orthoformate, aminolysis and hydroylsis afforded (±)-**2b**.

Scheme 9



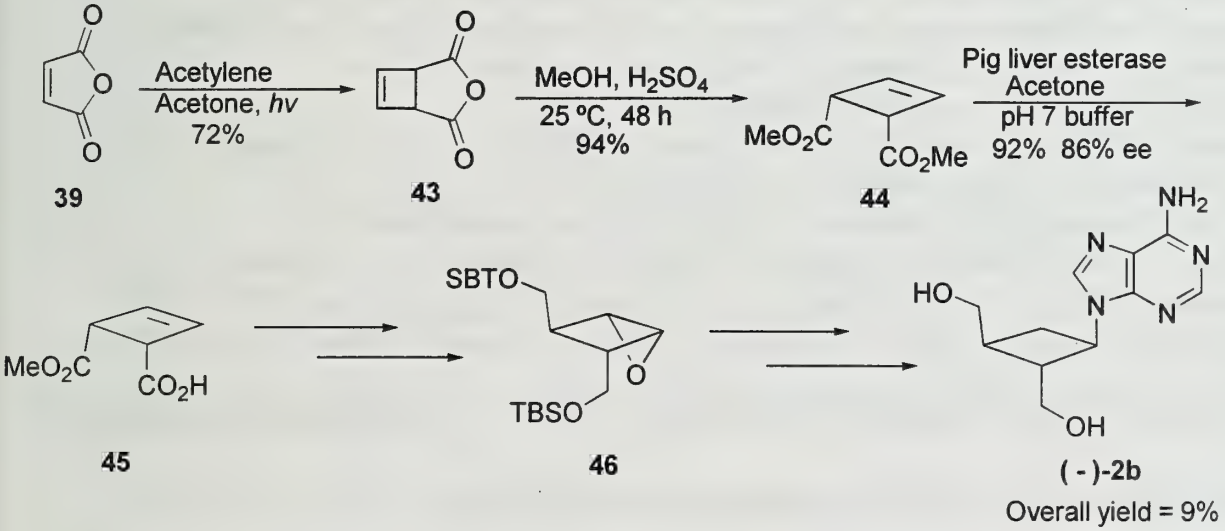
A shorter photochemical route to (±)-**2b** has been developed as outlined in Scheme 10.[16] Irradiation of anhydride **39** and acetate **40** followed by treatment with methanol afforded cyclobutane **41**. Cyclobutane **41** was then treated with adenine in the presence of potassium carbonate, followed by reduction with lithium aluminum hydride to yield (±)-**2b**.

Scheme 10



By combining a photochemical construction with an enzymatic resolution step, an enantioselective synthesis of (-)-**2b** has been accomplished (Scheme 11).[17] Irradiation of maleic anhydride with acetlylene afforded the cycloaddition product **43**, which upon hydrolysis and esterification yielded the diester **45**. The diester **45** was treated with an immobilized pig liver esterase (PLE) on a modified azalactone mixed polymer to afford the enantiomerically pure monoester **46** in a 92% yield with 86% ee.

Scheme 11



CONCLUSION

Carbocyclic nucleosides have been established as an important class of antiviral agents. The demand to produce enantiomerically pure carbocyclic analogues has resulted in the development of a wide variety of approaches. Highly enantioselective syntheses of carbocyclic nucleosides were described. Further development and optimization of synthetic strategies will result in a variety of enantiomerically pure carbocyclic nucleoside derivatives which could show activity against HIV and other virus.

REFERENCES

(1) Robins, M. J.; MacCoss, M. *Chemistry of Antitumor Agents,* Blackie and Son: U.K., **1990**, pg 261.

(2) Revankar, G. R.; Robins, R. K. *Antiviral Drug Development*, Plenum: New York, **1988**, pg 11.

(3) Vince, R.; Hua, M.; Brownell, J.; Daluge, S.; Lee, F.; Shannnon, W. M.; Lavelle, G. C.; Qualls, J.; Weislow, O. S.; Kiser, R.; Canonico, P. G.; Schultz, R. H.; Narayanan, V. L.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M.R. *Biochem. Biophys. Res. Commun.* **1988**, 156, 1046.

(4) Norbeck, D. W.; Kern, E.; Hiyashi, S.; Rosenbrook, W.; Sham, H.; Herrin, T.; Plattner, J. J.; Erickson, J.; Clement, J.; Swanson, R.; Shipkowitz, N.; Hardy, D.; Marsh, K.; Arnett, G.; Shannon, W.; Broder, S.; Mitsuya, H. *J. Med. Chem.* **1990**, 33, 1281.

(5) Biggadike, K.; Borthwick, A. *Tetrahedron.* **1992**, 48, 571.

(6) Agrofoglio, L.; Suhas, E.; Farese, A.; Condom, R.; Challand, R.; Earl, R. A.; Guedj, R. *Tetrahedron*, **1994**, 50, 10611.

(7) Evans, C. T.; Roberts, S. M.; Shoberu, K. A.; Sutherland, A. G. *J. Chem. Soc. Perkin Trans. 1* **1992**, 589.

(8) (a) Tanaka, M.; Norimine, Y.; Fujita, T.; Suemune, H. *J. Org. Chem* **1996**, 61, 6952.
(b) Tanaka, M.; Yoshioka, M.; Saka, K. *Tetrahedron: Asymmetry*, **1993**, 4, 981

(9) Hodgson, D. M.; Witherington, J.; Moloney, B. A. *J. Chem. Soc. Perkin Trans. 1* **1994**, 3373.

(10) Scheffold, R.; Troxler, T.; Hidbrand, S. *Helv. Chim. Acta* **1994**, 77, 1236.

(11) Vince, R.; Hua, M. *J. Med. Chem.* **1990**, 33, 17.

(12) (a) Trost, B. M.; Li, L.; Guile, S. D. *J. Am. Chem. Soc.* **1992**, 114, 8745. (b)Trost, B. M.; Kuo, G. H.; Benneche, T. *J. Am. Chem. Soc.* **1988**, 110, 621.

(13) Peel, M.R.; Johnson, M.R.; Sternbach, D. D. *J. Org. Chem.* **1991**, 56, 4990.

(14) Gharbaoui, T; Legraverend, M.; Ludwig, O.; Bisagni, E.; Aubertin, A.; Chertanova, L. *Tetrahedron* **1995**, 51, 1641. (b) Johnson, C. R.; Randall, L. D. *J. org. Chem* **1992**, 57, 594.

(15) Katagiri, N.; Nomura, M.; Sato, H.; Kaneko, C.; Yusa, K.; Tsuruo, T. *J. Med. Chem.* **1992**, 35, 1982.

(16) Somekawa, K.; Hara, R.; Kinnami, K.; Muraoka, F.; Suishu, T.; Shimo, T. *Chem. Lett.* **1995**, 407.

(17) Jung, M.; Sledeski, A. *J. Chem. Soc. Chem. Commun.* **1993**, 589.

STEREOSELECTIVE SYNTHESIS OF POLYSUBSTITUTED OLEFINS BY 1,2-METALLATE REARRANGEMENT AND 1,1-BIMETALLOALKENES

Reported by Rosanna Tedesco February 20, 1997.

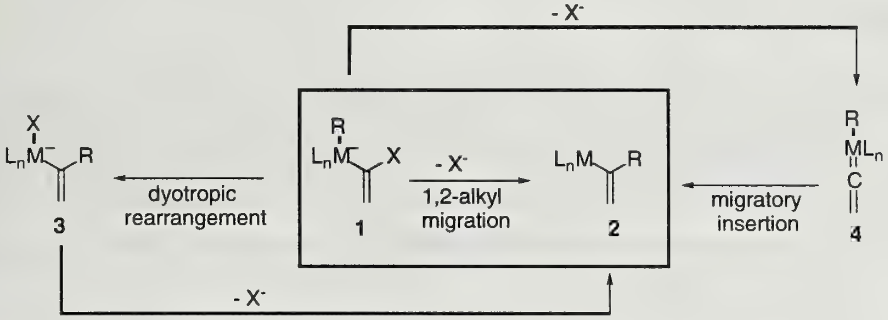
INTRODUCTION

Over the past thirty years, a variety of methods for the stereoselective synthesis of polysubstituted olefins has been developed, which provide high yields and stereoselectivities. Among the various strategies, the use of α-heteroalkenyl organometallic derivatives has received considerable attention. The first part of this report focuses on the 1,2-metallate rearrangement of these species, in particular the organocuprate rearrangement. This method has proven to be useful and generally applicable, provided the proper precursor and the appropriate set of experimental conditions, to the synthesis of various natural products, in particular macrolide polyketides. The second part of this report will present a brief description of bimetalloalkenes and their versatility in the stereospecific synthesis of polysubstituted olefins.

1,2-METALLATE REARRANGEMENT

The 1,2-metallate rearrangement can be defined as a carbon-carbon bond forming reaction in which an alkyl group R bound to the metal in alkenyl ate complex **1** undergoes a 1,2-shift to an electrophilic carbon attached to the same metal to give an alkenylmetallic product **2**. The transformation from **1** to **2**, as illustrated in Scheme 1, can occur directly with displacement of the heteroatom X by the alkyl group, or can proceed through the formation of an alkylidene carbene **4**, followed by migratory insertion of the alkyl group. Another possibility is the concerted interchange of the R and X group to give **3**, which by loss of X^- affords **2**.[1]

Scheme 1



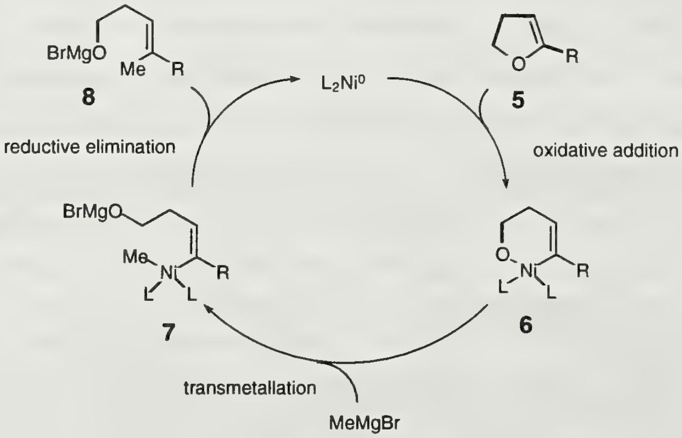
Nickel-Catalyzed Coupling of 5-Alkyl-2,3-Dihydrofurans and 6-Alkyl-3,4-2H-Pyrans with Grignard Reagents

The Ni(0)-catalyzed coupling of Grignard reagents, known as the Wenkert reaction, has long been used as an efficient method for the synthesis of disubstituted olefins. To extend this reaction to the

Copyright © 1997 by Rosanna Tedesco

stereoselective synthesis of trisubstituted alkenes, it would be necessary to prepare the α-heteroalkene precursor with stereocontrol. One method to achieve this requirement is by using 5-alkyl-2,3-dihydrofurans (**5**)[2] and 6-alkyl-3,4-2H-pyrans[3] as partners in the coupling reaction with Grignard reagents. The high degree of regio- and stereocontrol in this reaction results from the fixed double bond geometry of the cyclic enol ether precursor and the stereoselectivity of the Ni(0)-catalyzed coupling step, so that the alkyl group derived from the Grignard reagent becomes cis to the alcohol chain. Scheme 2 illustrates the catalytic cycle for this reaction.

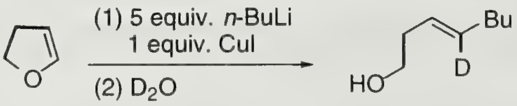
Scheme 2



This method is limited by several attributes of the starting materials necessary for the transformation. 5-Alkyl-2,3-dihydrofurans are rather sensitive compounds which are prone to the rearrangement of the double bond into the exocyclic position, giving compounds that are able to react with Grignard reagents in the presence of Ni(0), but do not lead to the desired products. 6-Alkyl-3,4-2H-pyrans are more stable and easier to prepare, but they only react with Grignard reagents devoid of β-hydrogens and present a diminished reactivity in the Ni(0)-catalyzed coupling reaction.

Organocuprate Rearrangement

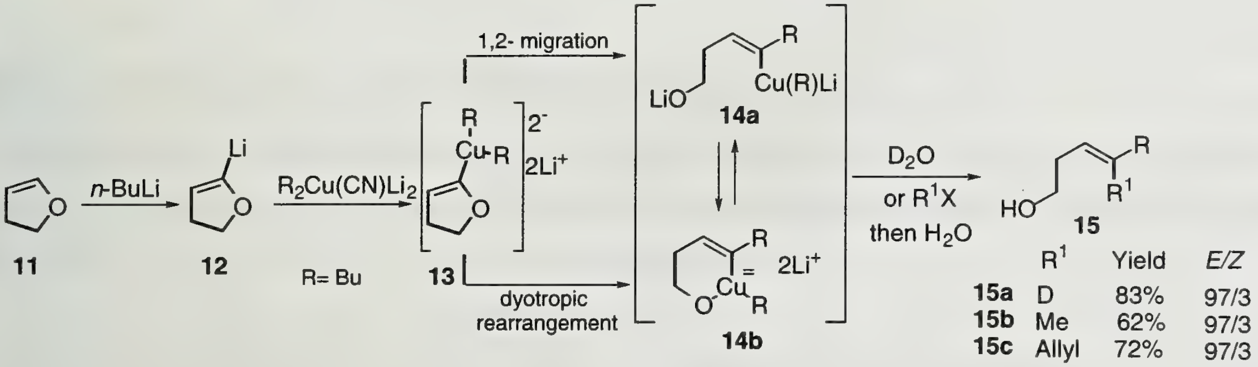
To improve the Wenkert reaction, Cu(I) was used in place of Ni(0) and a different selectivity was observed. Unlike the Ni(0)-catalyzed reaction, which proceeds with retention of configuration, the Cu(I)-catalyzed reaction seems to proceed with inversion of configuration. Dihydrofuran **9** reacts with *n*-BuLi in the presence of Cu(I) to give the *trans*-homoallylic alcohol **10**, whereas the Ni(0) catalyzed reaction gives the cis product.[1]



9 *trans*-**10**

The mechanistic dissimilarities of the two reactions were further characterized by some key observations: (1) no reaction was observed using 5-alkyl-2,3-dihydrofurans; (2) 5-lithiodihydrofurans gave the trans product when treated with *n*-BuLi in the presence of copper; (3) a quantitative incorporation of deuterium at C(4) was obtained upon quenching with D_2O. It has been proposed that the reaction proceeds through the initial formation of a higher order cuprate, as shown in Scheme 3. Dihydrofuran is metallated with *n*-BuLi, and the lithiated species then is allowed to react with a di-alkylcuprate generated *in situ* to form a higher order cuprate **13**. This species then undergoes 1,2-alkyl migration to give **14a**, or dyotropic rearrangement to give **14b**. Both of these proposed intermediates react with D_2O to give the deuterated product **15a** with retention of configuration. Addition of methyl iodide or allyl bromide to **14** affords the trisubstituted alkenes **15b** and **15c** with high stereoselectivity. The observation that Me_2S improves the reaction efficiency, together with the X-ray structure of higher order cuprates incorporating Me_2S as a stabilizing ligand,[4] confirm the involvement of a higher order cuprate as a key intermediate in the metallate rearrangement.[5]

Scheme 3

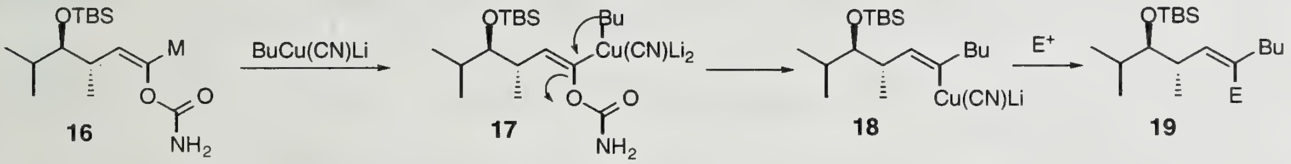


	R¹	Yield	*E/Z*
15a	D	83%	97/3
15b	Me	62%	97/3
15c	Allyl	72%	97/3

Acyclic Systems

Cu(I)-catalyzed, 1,2-alkyl migration reactions have also been studied using acyclic systems. (*Z*)-Enol carbamates (**16**, M=H), which are readily available with good stereocontrol by the method of Hoppe,[6] have found wide application as precursors in the stereocontrolled synthesis of trisubstituted alkenes. They can be easily metallated with *n*-BuLi in the presence of TMEDA; however, the lithio derivative is unstable at the temperature required for the rearrangement to occur, instead undergoing a Fritsch-Buttenberg-Wiechell rearrangement to an acetylene. The desired rearrangement can be achieved by Cu(I) transmetallation of the stannane **16** (M=$SnMe_3$).[1,7] The mechanism is illustrated in Scheme 4.

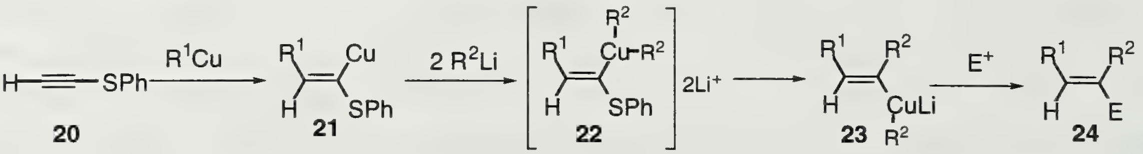
Scheme 4



Substrates for the 1,2-metallate rearrangement can also be obtained via carbocupration of phenylthioacetylenes (**20**, Scheme 5).[8] In the organocopper species **23**, derived from the acetylenic precursor, the cuprate unit has a trans relationship with the alkyl group R^1, whereas in the copper species obtained from (*Z*)-enol carbamates **18**, the copper moiety has a cis relationship with the alkyl group.

Addition of the electrophile gives the corresponding trisubstituted alkenes (**24**), with ≥ 99% stereoselectivity.

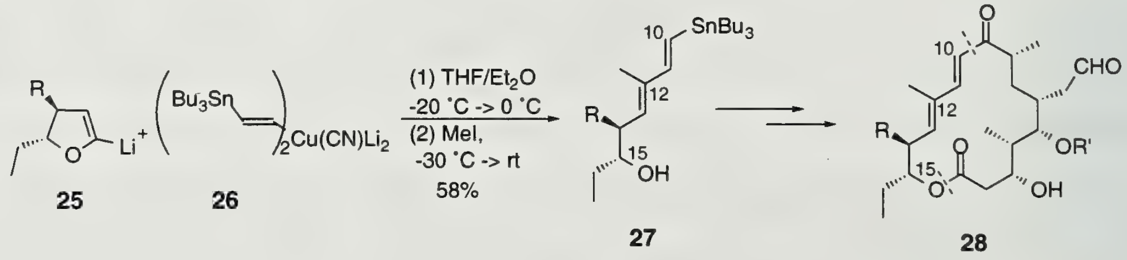
Scheme 5



Application of the 1,2-Metallate Rearrangement of Organocuprates in the Synthesis of Natural Products.

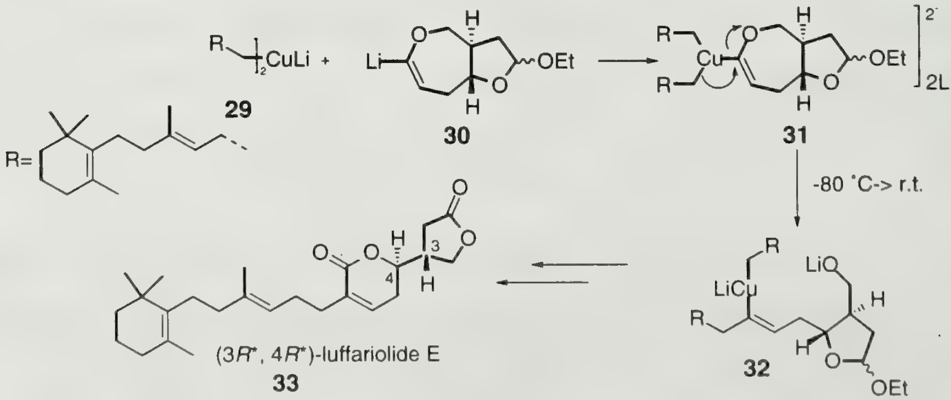
The strategies described above for the stereoselective synthesis of trisubstituted alkenes has found wide application in the synthesis of natural compounds in many macrolide polyketide fragments and terpenes. In the synthesis of the C(10)-C(15) fragment of desepoxy rosaramycin (**28**, R= H) and tylosin (**28**, R= OH), further elaboration of the method for the synthesis of the (*E*,*E*)-stannyl dienes and silyl dienes, have been reported.[7,9] Stannyl dienes were previously obtained from (*Z*)-enol carbamates but the use of 2,3-dihydrofurans has been shown to be more efficient. The western part of desepoxy rosaramycin (fragment C(10)-C(15), Scheme 6) was synthesized by addition of (stannyl)vinyl cyanocuprate **26** to dihydrofuran derivative **25**. The rearranged product was quenched with MeI, installing the C(12)-Me group. The (*E*,*E*)-stannyldiene **27** was obtained as a single isomer in 58% yield (Scheme 6).

Scheme 6



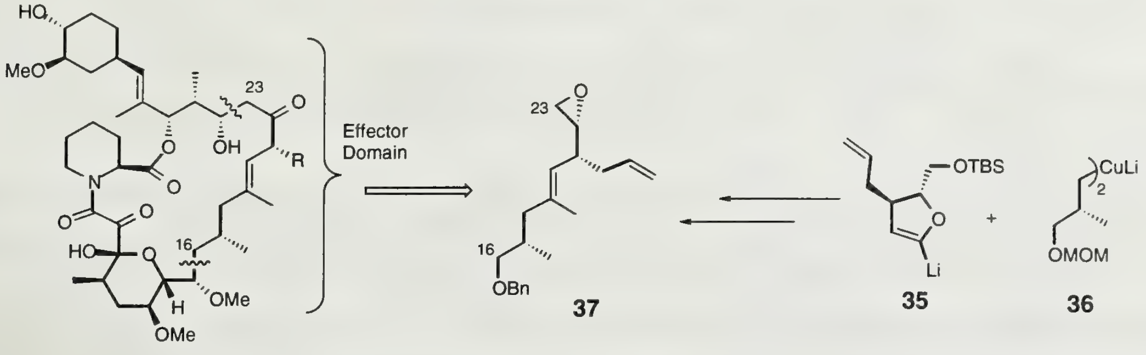
In the total synthesis of luffariolide E (**33**)[10,11] it was demonstrated that the higher-order cuprates derived from a seven-membered ring metallated enol ether, readily participate in the 1,2-metallate rearrangement with a reactivity and a stereoselectivity comparable to metallated dihydrofurans. (3*R**, 4*R**)-luffariolide E was synthesized starting from the trans bicyclic compound **31**.

Scheme 7



By use of the 1,2-cuprate rearrangement method, the effector domain of FK506 (**34**), a potent immunosuppressant, was obtained as a single geometric isomer (*E*).[12] In the previous syntheses of FK506, this fragment was always obtained as a mixture of *E* and *Z* isomers.[13,14]

Scheme 8

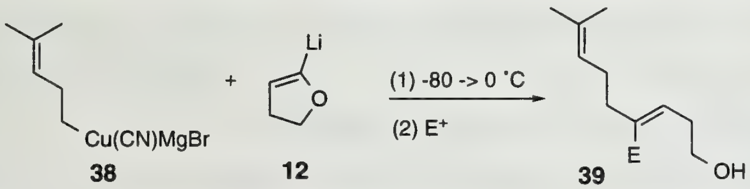


34 **37** **35** **36**

Metallate Rearrangement of Homoallylic Magnesiocuprate

Homoallylic cuprates derived from organolithium reagents fail to undergo the coupling due to competing β-elimination of CuH. The use of homoallylic, mixed magnesiocuprates[15] has surmounted this problem, making possible the transformation illustrated in Scheme 9.

Scheme 9



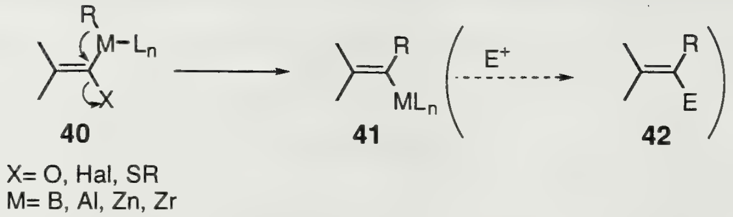
38 **12** **39**

The best results have been obtained by using tertiary and secondary alkyl Grignard reagents. In contrast with the lithiocuprates, magnesiocuprates give better yields with dihydropyran systems than with the dihydrofurans. Furthermore, the rearrangement of magnesiocuprates occurs at lower temperatures. Occasionally, the 1,2-metallate rearrangement of the higher order cyanocuprate derived from lithiated dihydropyrans requires temperatures at or above the ambient, in which case competing decomposition of the intermediate is observed, leading to low yields and complex product mixtures.

1,2-Shifts in Other 1-Hetero-1-alkenyl Metallate Complexes

A variety of heteroatom substituents and metals can participate in metallate rearrangement reactions.[1,16-18] 1-Bromoalkylborates were the first system in which this type of reactivity was observed and used in synthesis. α-Hetero-aluminum, -zinc, and -zirconium complexes can also afford polysubstituted olefins with reasonable stereoselectivity; however, their diminished reactivity has limited their utility.

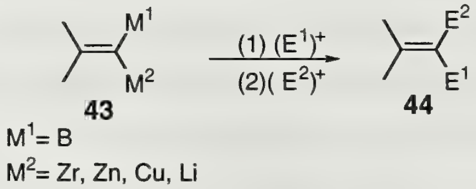
Scheme 10



1,1-BIMETALLOALKENES AS A SOURCE OF STEREODEFINED OLEFINS

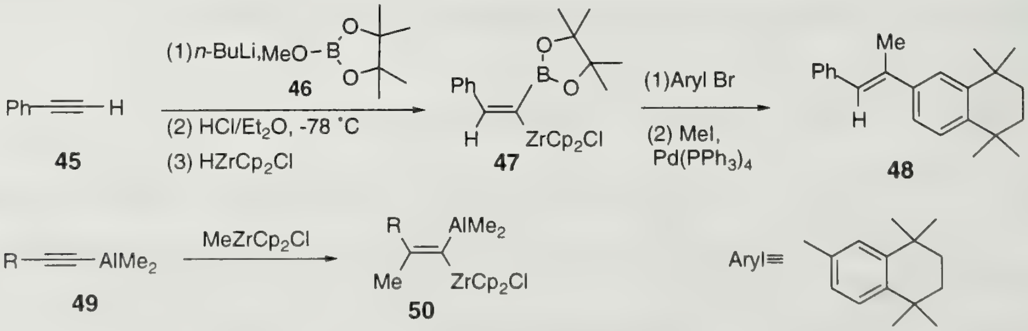
1,1-Bimetallic species have been utilized as alkylidene transfer reagents, and many synthetic applications can be found in the literature. Less attention has been devoted toward 1,1-bimetalloalkenes species as a source of stereodefined polysubstituted double bonds, despite their potential. The stereodifferentiation step results from the different reactivity of the two metals on the sp^2-dianionic derivative. An exception, in which the two metals are identical, arises from a regioselective intramolecular alkylation.[19]

Scheme 11



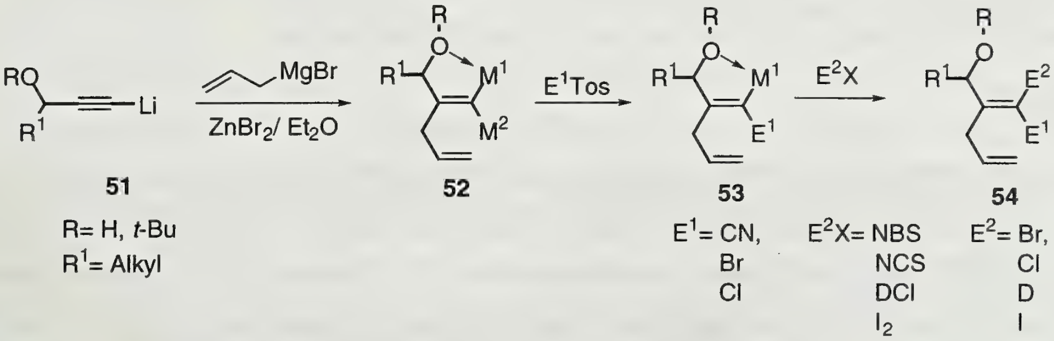
Boryl zirconocene 1,1-dimetalloalkenes **47** were successfully used in the synthesis of temarotene (**48**), a retinoid of great interest because of its low toxicity. Reaction of alkyne **45** with borate **46**, followed by hydrozirconation, gave the dimetallic species **47**.[20,21] These compounds are stable and are capable of undergoing selective and sequential reactions with a variety of electrophiles due to the considerably greater reactivity of the C-Zr bond compared to the C-B bond. Temarotene was synthesized efficiently and selectively, and in reasonable yield using this method.[22] 1,1-Zinc, boron and 1,1-copper, boron alkenyl bimetallics that can undergo transformations similar to the 1,1-zirconium, boron dimetallic species have also been obtained.[23] Aluminum zirconocene 1,1-dimetalloalkenes **50** (Scheme 12) were synthesized by methylzirconation of aluminum derivative **49**.[24]

Scheme 12



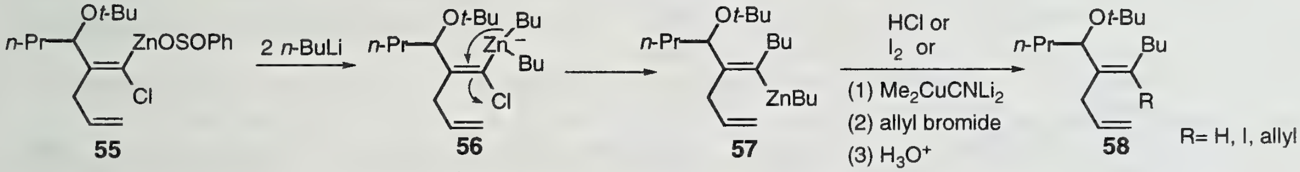
The reactivity of the two metals toward electrophiles can also be differentiated by internal chelation with a Lewis basic functional group.[25] When a lithiated propargyl alcohol or ether **51** is treated with allylmagnesium bromide and one equivalent of $ZnBr_2$, an organo-*gem*-bismetallic species **52** is generated.[25,26] The coordination of the oxygen atom to M^1 decreases its reactivity, and thus, the non-chelated metal M^2 reacts preferentially with electrophiles. Hydrolysis (or deuterolysis) of **53**, or reaction with other electrophiles affords tri or tetrasubstituted olefins as a single geometrical isomer.

Scheme 13



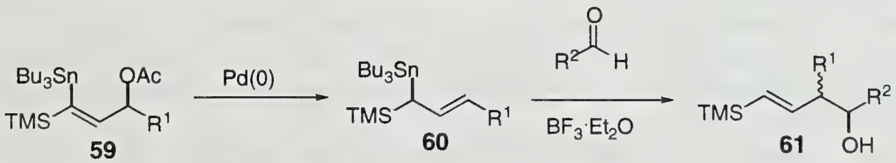
In the same context, the electrophilic behavior of the amphiphilic chlorocarbenoid species **55**, obtained by reaction of **52** with $PhSO_2Cl$, toward organometallic nucleophiles was studied. The addition of 2 equivalents of *n*-BuLi to **55** leads to the formation of carbenoid zincate **56**, which undergoes a smooth intramolecular nucleophilic substitution reaction, to give the vinylic organozincate **57**. This intermediate gives the corresponding olefins **58** in high stereochemical purity by treatment with HCl or I_2 or transmetallation followed by allylation.

Scheme 14



1,1-Stannyl, silyl alkenes **59** are precursors to allyl 1,1-bimetallic species **60** (Scheme 15). Reaction of **60** with aldehydes in the presence of a Lewis acid affords allylic alcohol **61** in good diastereomeric ratios. The formation of the *trans* allylic alcohol was always observed.[27]

Scheme 15



CONCLUSION

The methods presented above are valuable advances towards the goal of developing a general, stereoselective synthesis of polysubstituted olefins. The 1,2-metallate rearrangement has been shown to be applicable to the synthesis of natural products containing trisubstituted olefins, such as terpenes and polyketides. The functional groups tolerated or introduced allow elaboration toward the final product. The best results are obtained starting from cyclic enol ethers, which limits its applications to cases when these precursors are readily available, but such precursors are usually obtained with high stereoselectivity.

1,1-Bimetalloalkenes, although not yet widely utilized, are viable precursors for the selective introduction of substituents on a double bond. The different reactivity of the two sites allows the formation of olefins with excellent stereoselectivity. The starting substrates are generally easily prepared and a wide range of electrophiles can be introduced.

REFERENCES

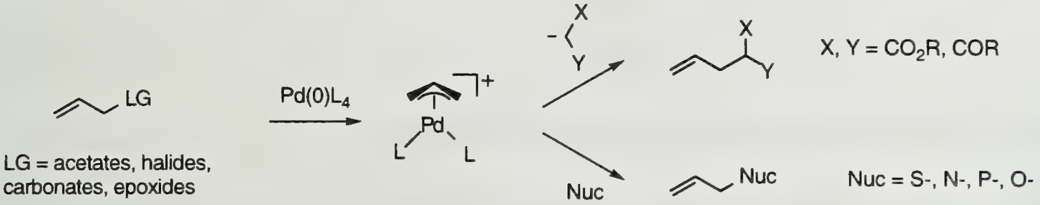
(1) Kocienski, P. J.; Barber, C. *Pure Appl. Chem.* **1990**, *62*, 1933.
(2) Kocienski, P. J.; Pritchard, M.; Wadman, S. N.; Whitby, R. J.; Yeates, C. L. *J. Chem. Soc., Perkin Trans. 1* **1992**, 3419.
(3) Ashworth, P. A.; Dixon, N. J.; Kocienski, P. J.; Wadman, S. N. *J. Chem. Soc., Perkin Trans. 1* **1992**, 3431.
(4) Olmstead, M. M.; Power, P. P. *J. Am. Chem. Soc.* **1990**, *112*, 8008.
(5) Ashworth, P.; Broadbelt, B.; Jankowski, P.; Kocienski, P.; Pimm, A.; Bell, R. *Synthesis* **1995**, 199.
(6) Zschage, O.; Hoppe, D. *Tetrahedron* **1992**, *48*, 5657.
(7) Fargeas, V.; Le Menez, P.; Berque, I.; Ardisson, J.; Pancrazi, A. *Tetrahedron* **1996**, *52*, 6613.
(8) Creton, I.; Marek, I.; Brasseur, D.; Jestin, J.-L.; Normant, J.-F. *Tetrahedron Lett.* **1994**, *35*, 6873.
(9) Le Menez, P.; Berque, I.; Fargeas, V.; Pancrazi, A.; Tran Huu Dau, M. E.; Ardisson, J. *SynLett* **1996**, 1125.
(10) Hareau-Vittini, G.; Kocienski, P.; Reid, W. *Synthesis* **1995**, 1007.
(11) Hareau-Vittini, G.; Kocienski, P. J. *SynLett* **1995**, 893.
(12) Jarowicki, K.; Kocienski, P.; Norris, S.; O'Shea, M.; Stocks, M. *Synthesis* **1995**, 195.
(13) Nakatsuka, M.; Ragan, J. A.; Sammakia, T.; Smith, D. B.; Uehling, D. E.; Schreiber, S. L. *J. Am. Chem. Soc.* **1990**, *112*, 5583.
(14) Jones, T. K.; Reamer, R. A.; Desmond, R.; Mills, S. G. *J. Am. Chem. Soc.* **1990**, *112*, 2998.
(15) Barber, C.; Bury, P.; Kocienski, P.; O'Shea, M. *J. Chem. Soc., Chem. Commun.* **1991**, 1595.
(16) Miller, J. A. *J. Org. Chem.* **1989**, *54*, 998.
(17) Negishi, E.; Akiyoshi, K. *J. Am. Chem. Soc.* **1988**, *110*, 646.
(18) Alexakis, A.; Hanaizi, J.; Jachiet, D.; Normant, J.-F. *Tetrahedron Lett.* **1990**, *31*, 1271.
(19) Negishi, E.; Sawada, H.; Tour, J. M.; Wei, Y. *J. Org. Chem.* **1988**, *53*, 913.
(20) Deloux, L.; Srebenik, L. *J. Org. Chem.* **1994**, *59*, 6871.
(21) Deloux, L.; Skrzypczak-Jankun, E.; Cheesman, B. V.; Srebnik, M. *J. Am. Chem. Soc.* **1994**, *116*, 10302.
(22) Deloux, L.; Srebnik, M.; Sabat, M. *J. Org. Chem.* **1995**, *60*, 3276.
(23) Waas, J. R.; Sidduri, A.; Knochel, P. *Tetrahedron Lett.* **1992**, *33*, 3717.
(24) Negishi, E.; Kondakov, D. Y.; Choueiry, D.; Kasai, K.; Takahashi, T. *J. Am. Chem. Soc.* **1996**, *118*, 9577.
(25) Creton, I.; Marek, I.; Normant, J.-F. *Tetrahedron Lett.* **1994**, *36*, 7451.
(26) Creton, I.; Marek, I.; Normant, J. F. *Synthesis* **1996**, 1499.
(27) Lautens, M.; Ben, R. N.; Delanghe, P. H. M. *Tetrahedron* **1996**, *52*, 7221.

ASYMMETRIC INDUCTION IN PALLADIUM-CATALYZED ALLYLIC SUBSTITUTION REACTIONS

Reported by Daniel J. Pippel February 24, 1997

INTRODUCTION

Palladium-catalyzed nucleophilic substitution of allylic substrates represents an important method for the formation of both carbon-carbon and carbon-heteroatom bonds at sp^3 centers.[1] These reactions proceed through cationic π-allyl intermediates which are square planar at palladium with respect to the ligands and two terminal carbons of the allyl unit. Compatibility with a wide range of leaving groups and nucleophiles renders the sequence particularly useful (eq 1). The development of enantioselective variants has provided a significant challenge due, in large part, to special requirements on the nature of the chiral inducers.[2-5] However, recent mechanistic understanding has facilitated the design of several ligands capable of producing highly enantioenriched products. This report describes the general catalytic cycle for palladium catalysis of allylic substitution, delineates the mode of asymmetric induction for three classes of ligands, and briefly highlights the utility of these ligands.



(1)

GENERAL CATALYTIC CYCLE

The catalytic cycle shown in Figure 1 has been established for the substitution of π-allyl palladium complexes by most heteroatom and stabilized carbon nucleophiles.[6-9]

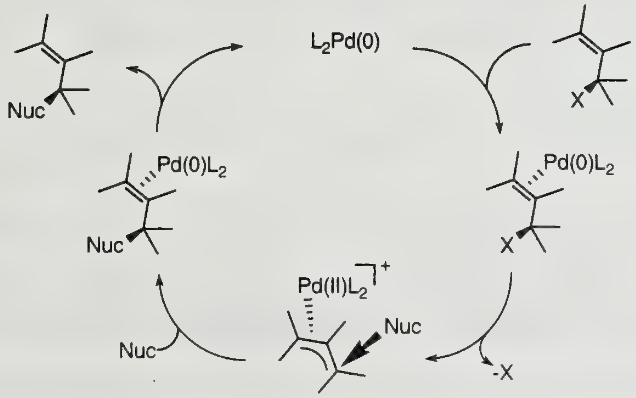


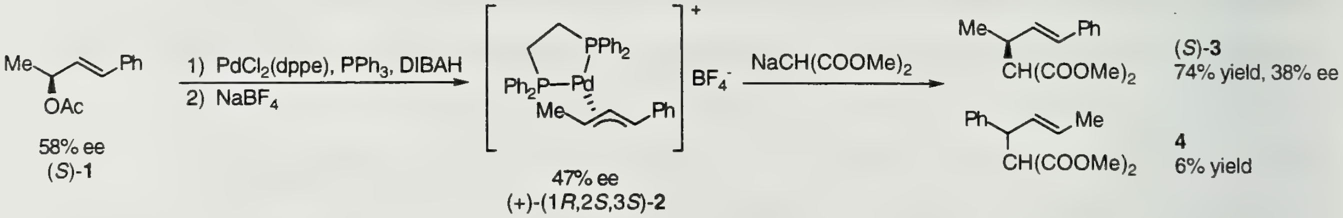
Figure 1. Palladium-catalyzed allylic substitution with stabilized nucleophiles.

Copyright © 1997 by Daniel J. Pippel

Metal-olefin complexation followed by dissociation of the nucleofuge from the π-face opposite the catalyst affords a cationic π-allyl-palladium(II) intermediate. Rate determining nucleophilic attack outside the coordination sphere of the metal and subsequent decomplexation regenerates the catalyst and provides allylic products in high yields. Delivery of hard nucleophiles such as Grignard reagents or alkylzincs occurs via initial coordination with palladium; these processes have seen only limited investigation and will not be considered here.[2]

As shown in Scheme 1, both bond-breaking and bond-making occur on the π-allyl face distal to palladium and its attendant ligands.[6] Treatment of an enantiomerically enriched allylic acetate (*S*)-**1** with a solution presumed to contain Pd(dppe)(PPh_3) (dppe = 1,2-bis(diphenylphosphino)ethane) yielded the π-allyl-palladium complex (+)-(1*R*,2*S*,3*S*)-**2**. Comparison with authentic (–)-(1*S*,2*R*,3*R*)-**2** indicated that the intermediate was formed stereoselectively with 81% inversion. Alkylation to give the major regioisomeric product (*S*)-**3** also proceeded via inversion. Thus, an inversion-inversion process (net retention) characterizes these transformations.

Scheme 1



MECHANISTIC CONSIDERATIONS IN LIGAND DESIGN

The doubly invertive mechanism for palladium-catalyzed, allylic substitution reactions spatially removes the chiral ligands from the reaction center, restricting their ability to control product configurations. The multiple sources of potential asymmetric induction also complicate ligand design. Depending on the substrate, any event in the catalytic cycle other than final decomplexation could constitute the enantiodetermining step (Figure 2). Many workers have focused on the design of ligands which promote regioselective attack on meso intermediates (iii). Although the utility of these ligands often extends to other scenarios, only mode iii will be discussed here.

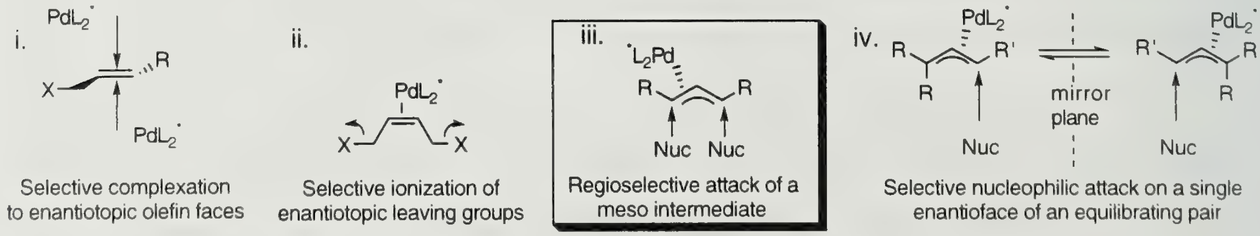
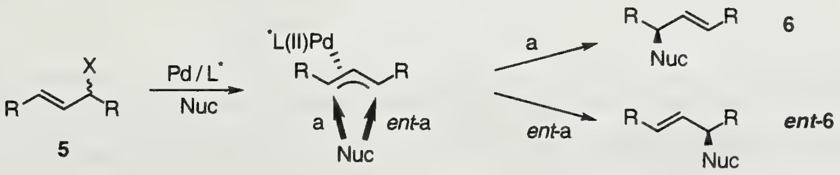

i.
PdL2*
X
R
PdL2*
Selective complexation to enantiotopic olefin faces
ii.
PdL2*
X
X
Selective ionization of enantiotopic leaving groups
iii.
*L2Pd
R
R
Nuc
Nuc
Regioselective attack of a meso intermediate
iv.
PdL2*
R
R'
R
Nuc
mirror plane
PdL2*
R'
R
R
Nuc
Selective nucleophilic attack on a single enantioface of an equilibrating pair

Figure 2. Enantiodetermining events in palladium-catalyzed allylic substitution reactions.

As shown in Scheme 2, substituted allylic substrates of type **5** form meso π-allyl intermediates (the generally accepted stereochemical nomenclature refers only to the allyl moiety). If the incoming nucleophile chooses preferentially between the two enantiotopic termini, an enantioenriched product, **6** or ***ent*-6**, results. For a given substrate-ligand combination, the sense but not always the extent of asymmetric induction is independent of nucleophile and leaving group.[10,11]

Scheme 2



With meso π-allyl complexes, two η^3-η^1-η^3 isomerization pathways can be operative (Figure 3, I and II).[2] In case I, an initial change in hapticity affords an η^1 complex which can rotate around the carbon-carbon single bond. Reformation of the η^3 complex results in a formal syn-anti isomerization with concurrent facial migration of palladium. Neither diphenyl nor cyclic substrates undergo this process and are often employed. Diphenyl π-allyl complexes exist only as syn-syn isomers due to steric effects; cylic complexes exist as anti-anti isomers due to geometrical constraints. In case II, formation of the η^1 species, rotation around the C-Pd bond, and reformation of the η^3 complex manifests itself as apparent rotation of the π-allyl moiety. The process is degenerate when ligands possess C_2 symmetry.

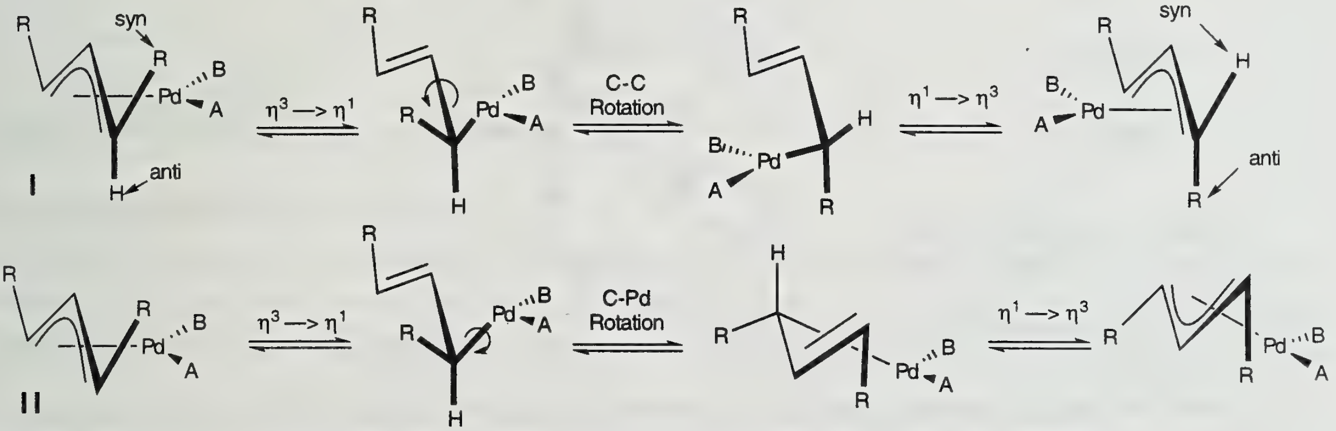


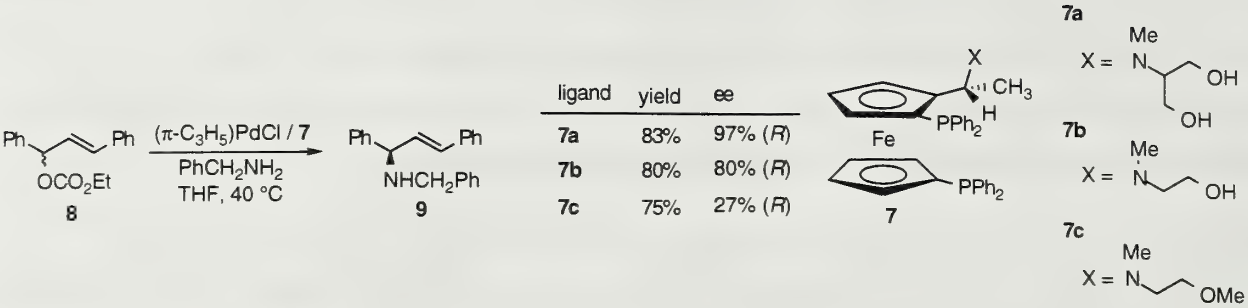
Figure 3. η^3-η^1-η^3 Pathways for syn-anti isomerization (I) and apparent π-allyl rotation (II).

Traditional chiral ligands such as (+)-DIOP were not successful for **5** (~20% ee when R = Ph),[12] and new ligands were designed. Three main categories will be considered: interacting tethers, chiral pockets, and electronic differentiation of termini.

Interacting Tethers

Hayashi's ferrocenylphosphine ligands, **7**, have a functional group tethered to the ferrocenyl ring (Scheme 3).[13-15] Benzylamine reacts with diphenyl carbonate **8** in the presence of a palladium catalyst

Scheme 3



ligand	yield	ee
7a	83%	97% (*R*)
7b	80%	80% (*R*)
7c	75%	27% (*R*)

and ligands **7a** or **7b** to provide products of high enantiomeric excess.[14] Presumably the distal hydroxy groups of these ligands participate electrostatically with the nucleophile, leading it almost exclusively to a single terminus (Figure 4). The corresponding ligand **7c** bearing a methoxy substituent on the pendant side chain does not initiate the same high degree of selectivity. This suggests the possibility of hydrogen bond formation between the hydroxyl group and incoming nucleophile. The mechanistic hypothesis is further corroborated with crystallographic data for the unsubstituted π-allylpalladium complex **10** which contains ligand **7b** (Figure 4). Specifically, the terminal hydroxy group orients itself in the general vicinity of the allyl group with greater proximity to one terminus: 3.36 and 5.24 Å from the two terminal carbons. However, other studies (*vide infra*) show significant structural differences between complexes containing substituted versus unsubstituted π-allyl units.

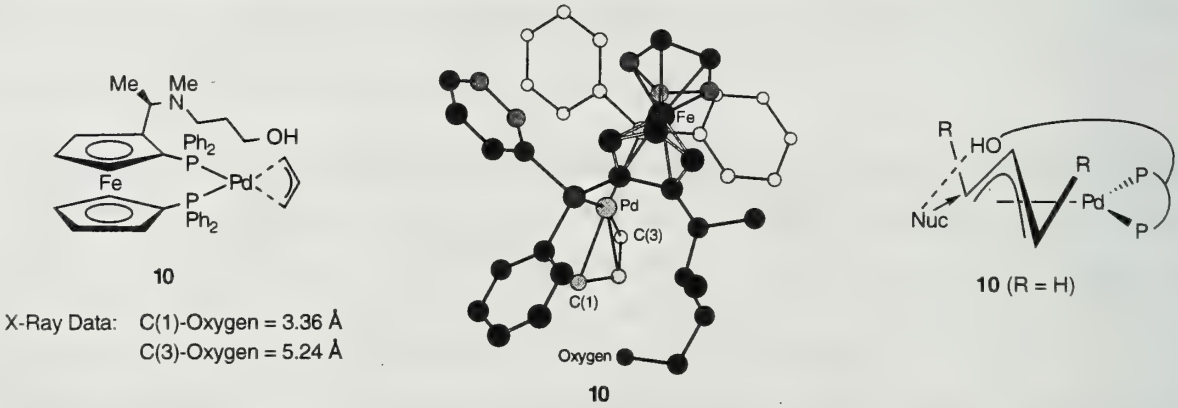


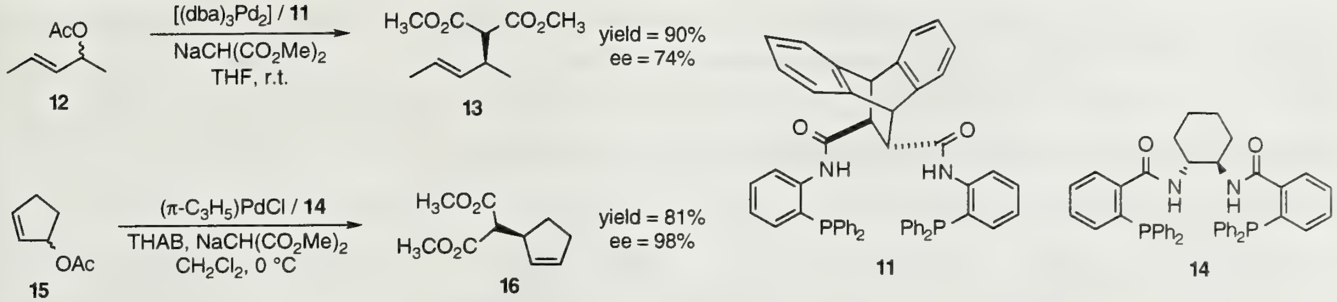
Figure 4. X-ray structure and schematic of **10** indicating possible mode of action for ferrocenyl ligands.

^{31}P NMR studies demonstrated that the π-allyl palladium complex of **7b** exists as two rotamers (20:1) in solution at 0 °C (Figure 3, II). The thermodynamically more stable rotamer is assumed to lead to the lower energy transition state for substitution.

The Chiral Pocket

An alternative concept in ligand design has been extensively developed by Trost.[16-21] In conjunction with a palladium catalyst and chiral amides **11** and **14**, dimethyl malonate reacts with the acyclic **12** and cyclic **15** to give enantioenriched products (Scheme 4).[19,22] These ligands minimize the degrees of freedom in the ligand-metal-π-allyl complex. Increased rigidity and symmetry should favor a single transition structure for the enantiodetermining step, resulting in enantioselective product

Scheme 4



formation. Amide linkages form an inflexible C_2 symmetric chiral backbone that effects a conformational bias in the two diphenylphosphino groups. Edge-face interactions between phenyl groups give rise to a "chiral propeller" which surrounds the substrate. Chelation of palladium by **11** or **14** forms a 13-membered ring (rigorously established as the reactive intermediate),[23] effectively increasing the bite angle, θ, and forcing greater interaction between the chiral pocket and attached substrate (Figure 5). The exact nature of the interaction between substrate and ligand remains unclear; however, a recent crystal structure of an unsubstituted π-allyl Pd complex formed from **11** reveals a bite angle of 110.5°. Both solid state and solution data indicate that the complex forms without symmetry, casting doubt on Trost's initial hypothesis regarding the importance of C_2 symmetry.

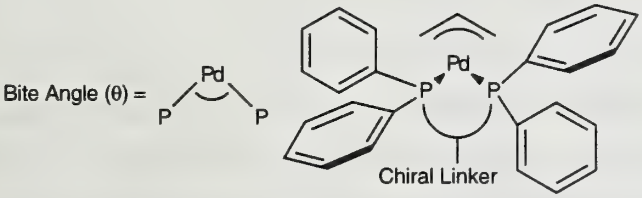
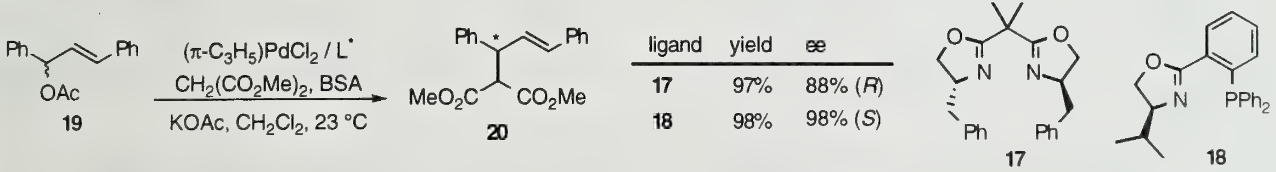


Figure 5. Phenyl group edge-face interactions which form the chiral pocket.

Electronic Differentiation of Termini

A third motif in ligand design, pioneered by Pfaltz, Helmchen and Williams,[24-27] exploits the ability of certain ligands to electronically differentiate the two allylic termini of a π-allyl-palladium complex. The asymmetric substitution of diphenyl acetate **19** catalyzed by a palladium complex with bis(oxazoline) ligand **17** (Scheme 5) provides a mechanistically clear example of this phenomenon.[28,29] A crystal structure of the π-allyl intermediate **22** reveals a bias for nucleophilic attack at one of the two enantiotopic termini (Figure 6). Specifically, C(1) lies significantly further from the central Pd, 2.17 Å, relative to C(3), 2.12 Å. The analogous, unsubstituted π-allyl Pd complex **21** shows no difference in

Scheme 5



ligand	yield	ee
17	97%	88% (*R*)
18	98%	98% (*S*)

bond lengths between Pd and the allylic termini; this suggests that the electronic bias in the substituted case results from steric interactions between the chiral ligand and the C(1) phenyl group. In addition, a downfield ^{13}C NMR shift is thought to demonstrate a greater partial positive charge on C(1), again relative to C(3). These factors favor nucleophilic attack at C(1), breaking the less stable C(1)-Pd bond, releasing strain and ultimately forming enantiomerically enriched **20**.

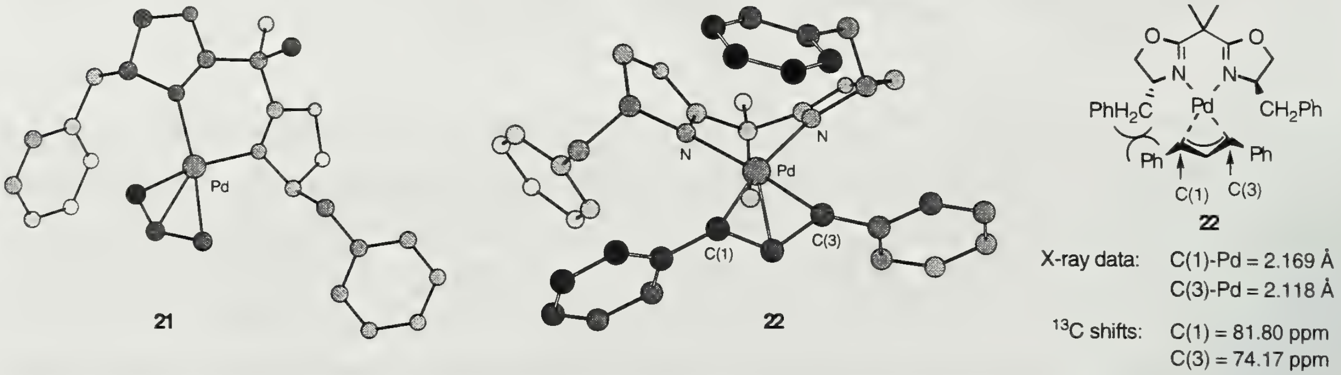


Figure 6. Crystal structures of unsubstituted (**21**) and substituted (**22**) π-allyl complexes.

Phosphinoaryloxazoline ligand **18** electronically differentiates the π-allyl termini of the palladium complex formed from **19** and induces regioselective attack by dimethyl malonate (Scheme 5).[30-32] However, in this case, the desymmetrization stems not from steric effects, but from the *trans* influence. Backbonding, or π-accepting ligands, including phosphines, utilize empty π* orbitals to accept electron density from filled d orbitals of the metal. σ-Donating ligands, such as nitrogen, add electron density to the metal via overlap between filled sp^n and vacant dsp hybrid orbitals. In a square planar array, ligands *trans* to π-acceptors are predicted to be less tightly held, while ligands *trans* to σ-donors are predicted to be more tightly held. An X-ray structure of the diphenyl substituted π-allyl palladium complex of **18** reveals bond lengths which are in accord with this prediction (Figure 7). The *exo* and *endo* isomers equilibrate to an 8 : 1 ratio at room temperature; this process occurs 50 times faster than nucleophilic attack. The major enantiomer of the product presumably results from reaction with the thermodynamically more stable *exo* isomer at the site of the longer C(1)-Pd bond in a Curtin-Hammett controlled reaction.

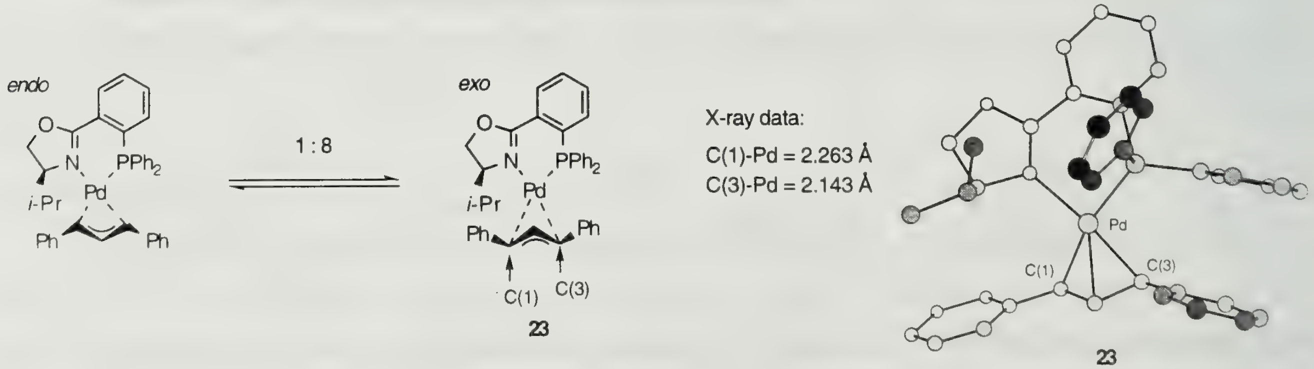
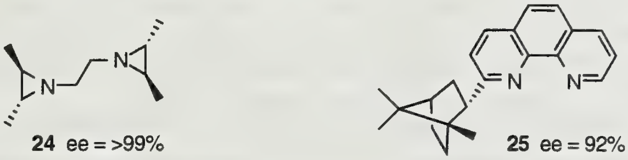


Figure 7. Schematic and X-ray structure for π–diphenylallyl complex **23**.

Other Ligands

A multitude of other ligands have been successfully employed in these reactions; however the exact mode of action is not always clear.[27,33-38] Figure 8 shows the recently reported C_2 symmetric bis(aziridine) **24**[39] and chiral phenanthroline **25**.[40] For both ligands, molecular mechanics calculations of their corresponding π-allyl palladium intermediates provide optimized structures which can be used in a predictive sense. Although these calculations suffer from complications arising in Curtin-Hammett situations and the relatively undeveloped state of the parameter sets, it appears likely that such computational studies will play an increasing role in future development of ligands.



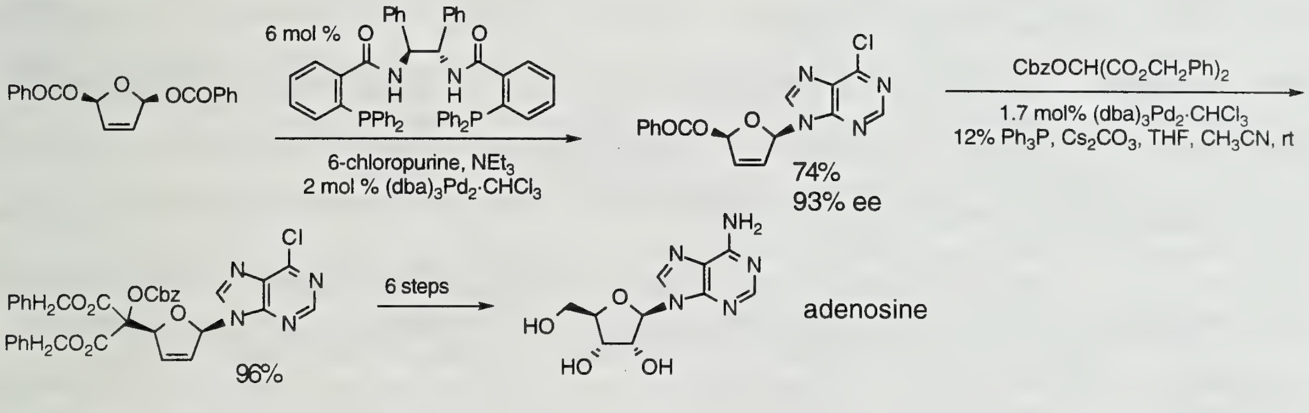
24 ee = >99% 25 ee = 92%

Figure 8. Enantiomeric excess of **20** using ligands **24** and **25** in reaction of **19** (Scheme 5).

UTILITY IN OTHER MODES OF ASYMMETRIC INDUCTION

Promotion of selective attack at a single enantiotopic terminus is not the only possible route to asymmetric induction in palladium-catalyzed, allylic substitution reactions (*vide supra*). Ligands which are successful with meso intermediates have also been used in controlling the events of other processes such as desymmetrization (Figure 1, ii). The synthesis of adenosine using a chiral-pocket-type ligand provides one example (Scheme 6).[18] The allylic substitution is highlighted by mild conditions, preservation of functionality, C-heteroatom bond formation, and a rapid increase in complexity--all of which are characteristic of these catalytic, asymmetric, allylic substitution reactions.

Scheme 6



CONCLUSIONS

The realization of asymmetric, palladium-catalyzed, allylic substitution reactions required an understanding of several inherent mechanistic complexities which thwarted early attempts at enantioselective variants. Design, testing, and mechanistic rationalization of new classes of ligands continues to provide fertile ground for much research.

REFERENCES

(1) Hegedus, L. S. In *Palladium in Organic Synthesis*; Schlosser, M., Ed.; John Wiley & Sons: New York, 1994, p 383.

(2) Trost, B. M.; Van Vranken, D. L. *Chem. Rev.* **1996**, *96*, 395.

(3) Frost, C. G.; Howarth, J.; Williams, J. M. J. *Tetrahedron: Asymmetry* **1992**, *9*, 1089.

(4) Consiglio, G.; Waymouth, R. M. *Chem. Rev.* **1989**, *89*, 257.

(5) Hayashi, T. In *Asymmetric Carbon-Carbon Bond Forming Reactions*; Ojima, I., Ed.; VCH Publishers, Inc.: New York, 1993, p 323.

(6) Hayashi, T.; Hagihara, T.; Konishi, M.; Kumada, M. *J. Am. Chem. Soc.* **1983**, *105*, 7767.

(7) Trost, B. M.; Strege, P. E.; Weber, L.; Fullerton, T. J.; Dietsche, T. J. *J. Am. Chem. Soc.* **1978**, *100*, 3407.

(8) Trost, B. M.; Weber, L.; Strege, P. E.; Fullerton, T. J.; Dietsche, T. J. *J. Am. Chem. Soc.* **1978**, *100*, 3416.

(9) Mackenzie, P. B.; Whelan, J.; Bosnich, B. *J. Am. Chem. Soc.* **1985**, *107*, 2046.

(10) Trost, B. M.; Bunt, R. C. *J. Am. Chem. Soc.* **1994**, *116*, 4089.

(11) Trost, B. M.; Bunt, R. C. *J. Am. Chem. Soc.* **1996**, *118*, 235.

(12) Reiser, O. *Angew. Chem. Int. Ed.* **1993**, *32*, 547.

(13) Hayashi, T.; Yamamoto, A.; Hagihara, T.; Ito, Y. *Tetrahedron Lett.* **1986**, *27*, 191.

(14) Hayashi, T.; Yamamoto, A.; Ito, Y.; Nishioka, E.; Miura, H.; Yanagi, K. *J. Am. Chem. Soc.* **1989**, *111*, 6301.

(15) Hayashi, T. *Pure & Appl. Chem.* **1988**, *60*, 7.

(16) Trost, B. M.; Van Vranken, D. L. *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 228.

(17) Trost, B. M.; Van Vranken, D. L.; Bingel, C. *J. Am. Chem. Soc.* **1992**, *114*, 9327.

(18) Trost, B. M. *Acc. Chem. Res.* **1996**, *29*, 355.

(19) Trost, B. M.; Breit, B.; Peukert, S.; Zambrano, J.; Ziller, J. W. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2386.

(20) Trost, B. M.; Bunt, R. C. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 99.

(21) Trost, B. M.; Murphy, D. J. *Organometallics* **1985**, *4*, 1143.

(22) Trost, B. M.; Organ, M. G. *J. Am. Chem. Soc.* **1994**, *116*, 10320.

(23) Trost, B. M.; Breit, B.; Organ, M. G.; *Tetrahedron Lett.* **1994**, *35*, 5817.

(24) Sprinz, J.; Helmchen, G. *Tetrahedron Lett.* **1993**, *34*, 1769.

(25) Pfaltz, A. *Acc. Chem. Res.* **1993**, *26*, 339.

(26) von Matt, P.; Loiseleur, O.; Koch, G.; Pfaltz, A.; Lefeber, C.; Feucht, T.; Helmchen, G. *Tetrahedron: Asymmetry* **1994**, *5*, 573.

(27) Allen, J. V.; Coote, S. J.; Dawson, G. J.; Frost, C. G.; Martin, C. J.; Williams, J. M. J. *J. Chem. Soc. Perkin Trans. 1* **1994**, *15*, 2065.

(28) Zehnder, M.; Neuburger, M.; von Matt, P.; Pfaltz, A. *Acta Crystallogr., Sect. C* **1995**, *51*, 1109.

(29) von Matt, P.; Lloyd-Jones, G. C.; Minidis, A. B. E.; Pfaltz, A.; Macko, L.; Neuburger, M.; Zehnder, M.; Rüegger, H.; Pregosin, P. S. *Helv. Chim. Acta* **1995**, *78*, 265.

(30) von Matt, P.; Pflatz, A. *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 566.

(31) Sprinz, J.; Kiefer, M.; Helmchen, G.; Reggelin, M.; Huttner, G.; Walter, O.; Zsolnai, L. *Tetrahedron Lett.* **1994**, *35*, 1523.

(32) Dawson, G. J.; Williams, J. M. J. *Tetrahedron Lett.* **1995**, *36*, 461.

(33) Hayashi, Y.; Sakai, H.; Kaneta, N.; Uemura, M. *J. Organomet. Chem.* **1995**, *503*, 143.

(34) Yamaguchi, M.; Yabuke, M.; Yamagishi, T.; Sakai, K.; Tsubomura, T. *Chem. Lett.* **1996**, 241.

(35) Togni, A.; Burckhardt, U.; Gramlich, V.; Pregosin, P. S.; Salzmann, R. *J. Am. Chem. Soc.* **1996**, *118*, 1031.

(36) Hayashi, T.; Ohno, A.; Lu, S.; Matsumoto, Y.; Fukuyo, E.; Yanagi, K. *J. Am. Chem. Soc.* **1994**, *116*, 4221.

(37) Chesney, A.; Bryce, M. R. *Tetrahedron: Asymmetry* **1996**, *7*, 3247.

(38) Kubota, H.; Koga, K. *Tetrahedron Lett.* **1994**, *35*, 6689.

(39) Andersson, P. G.; Harden, A.; Tanner, D.; Norrby, P. *Chem. Eur. J.* **1995**, *1*, 12.

(40) Peña-Cabrera, E.; Norrby, P.; Sjögren, M.; Vitagliano, A.; De Felice, V.; Oslob, J.; Ishii, S.; O'Neill, D.; Åkermark, B.; Helquist, P. *J. Am. Chem. Soc.* **1996**, *118*, 4299.

STUDIES ON THE BIOSYNTHESIS OF PACLITAXEL

Reported by Virginia K. B. Lehmann **February 27, 1997**

INTRODUCTION

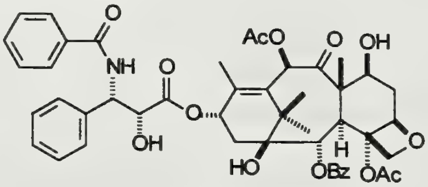
Cancer is predicted to overtake heart disease as the leading cause of death early in the next century.[1] Currently, refractory ovarian cancer and metastic breast cancer claim the lives of 60,000 women each year.[2] Both types of cancer have historically been hard to treat. However, new hope for successful treatment exists due to the plant natural product paclitaxel, Taxol®, **(1)** Figure 1. Originally isolated in the 1960s from the Pacific yew (*Taxus brevifolia*), its structure was established in 1971, and its mode of action was determined in 1979.[3-6] Paclitaxel prevents cytoplasmic microtubule depolymerization, thus interrupting the G_2 and M phases of the cell cycle. The FDA approved **1** for use in treating refractory ovarian cancer in 1992 and metastic breast cancer in 1994.[1] Clinical studies also show promise in treating other forms of cancer including lung, bladder, esophagus, endometrium, and cervical cancer in addition to lymphomas and Kaposi's sarcoma. Paclitaxel promises to be a powerful weapon in the battle against cancer.

As with many natural products, availability of adequate amounts of material for treatment remains a drawback to widespread use. Originally the only source was the bark of the Pacific yew.[3] This created environmental problems as the Pacific yew is a slow growing tree, and the tree is killed in harvesting the bark. One kilogram of **1** requires an average of 2500 mature (100 year old) trees, and it is predicted that 300 kg may be needed per year as **1** is approved for use in treating more forms of cancer.[2] Although two total syntheses have been achieved, the routes are too low-yielding to be viable.[7-9] Current production involves the semisynthesis from 10-deacetylbaccatin-III or baccatin-III isolated from the stems and needles of *Taxus* spp.[1] Whereas this method does not kill the tree, it still suffers from the low natural abundance of the semisynthetic precursor in the tree.

The most promising routes to **1** are through cell culturing of *Taxus* spp. and microbial fermentation of paclitaxel-producing fungi. A cell culture producing **1** at 0.6 % dry weight has been reported.[10] Futher, paclitaxel-producing fungi, *Taxomyces andeanae* and *Pestalotiopsis microspora*, have been identified with *P. microspora* having strains producing up to 60 μg/L in the culture.[2,11-14] Genetic manipulation could increase production of **1** in both cell cultures and microbial fermentations through

Copyright © 1997 by Virginia K. B. Lehmann

overexpression. To achieve these genetic manipulations, an understanding of the biosynthesis of **1** is needed. This report will discuss recent advances in the understanding of paclitaxel biosynthesis.



1

Figure 1. The structure of paclitaxel.

BIOSYNTHESIS OF PACLITAXEL BY FUNGI

Biosynthetic studies on fungal-produced **1** have been limited to precursor feeding studies. For *T. andreanae* and *P. microspora*, sodium [1-^{14}C]acetate and [U-^{14}C]phenylalanine were incorporated into **1**.[12,14] [2-^{14}C]Mevalonolactone and [U-^{14}C]glucose were not incorporated into **1** from *T. andreanae*, and sodium [7-^{14}C]benzoate and [U-^{14}C]leucine were not incorporated into **1** from *P. microspora*. The work has not yet elucidated the individual steps of the biosynthesis as it has for *Taxus*-produced **1**.

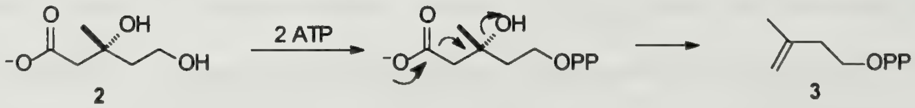
BIOSYNTHESIS OF PACLITAXEL BY *TAXUS* SPP.

The biosynthetic work on paclitaxel production by *Taxus* spp. has led to the elucidation of most of the pathway. The steps from isopentenyl pyrophosphate formation to the first hydroxylation and from 10-deacetylbaccatin-III to paclitaxel have been examined.

Formation of Isopentenyl Pyrophosphate

Paclitaxel contains a highly functionalized diterpenoid skeleton with a phenylalanine derived side chain. Diterpenes are formed from geranylgeranyl pyrophosphate (GGPP), which in turn is the condensation product of three units of isopentenyl pyrophosphate (IPP) and one unit of dimethylallyl pyrophosphate (DMAPP). One pathway for the formation of IPP and its isomer DMAPP is through mevalonic acid (MVA), Scheme 1. Mevalonate **(2)** is doubly phosphorylated by two molecules of ATP; it then undergoes decarboxylation to form IPP **(3)**.

Scheme 1

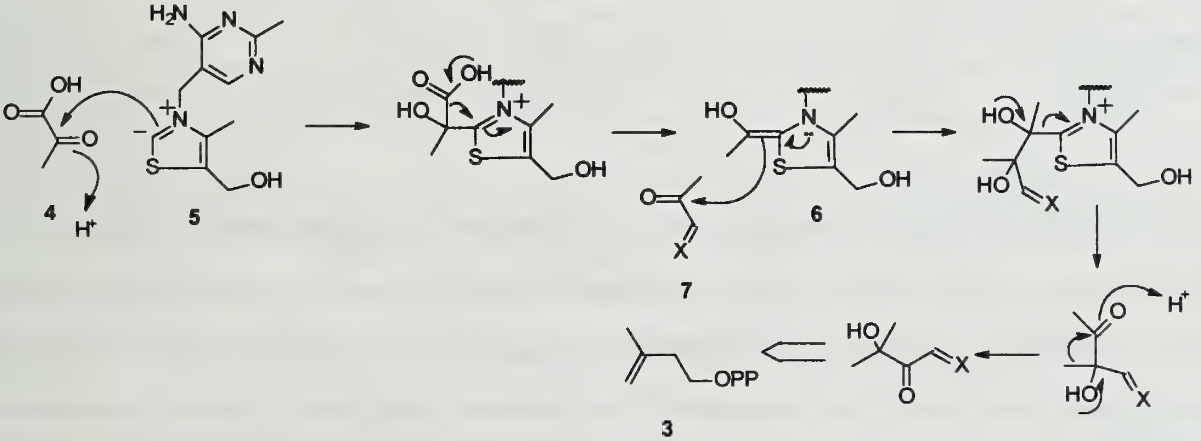


Initial feeding studies using cell cultures supported this pathway for *Taxus* spp. When [^{3}H]acetate, [5-^{3}H]mevalonate, and [2-^{14}C]mevalonate were fed to cell free cultures from ground needles and stems from *Taxus canadensis*, incorporation of 0.02-0.12% into **1** was found.[15] On the basis of levels of incorporation, the authors claimed the diterpenoid part was derived from MVA. No chemical degradations were carried out to ascertain the location of the label in the compound, so it is impossible to definitely state where the label was incorporated.

When [1-^{13}C]acetate was fed to freshly harvested inner bark of *T. brevifolia*, it was found to incorporate into the acetyl side chains as well as the diterpenoid portion.[16] Sodium [1-^{14}C]acetate, [2-^{14}C]mevalonolactone, [U-^{14}C]glucose, [1-^{14}C]leucine, and [U-^{14}C]phenylalanine were also fed, with leucine and phenylalanine showing the best incorporation into **1**. Basic hydrolysis showed that over 90% of the label for mevalonolactone and glucose were located in the acetyl groups and side chain, over 75% for leucine and phenylalanine, and 66% for acetate. The remaining activity was located in the taxane carbon skeleton.

However in 1996, it was reported that mevalonate was not incorporated into the diterpenoid portion of **1**, but rather **3** was formed through the Rohmer pathway, Scheme 2.[17] The Rohmer pathway proposes the activation of pyruvate **(4)** by thiamine **(5)** to form an activated acetaldehyde **(6)** which undergoes condensation with a compound **(7)** derived from dihydroxyacetone, followed by a rearrangement which includes a methyl shift.[18] It has been reported that in *Ginkgo biloba*, ginkgolides have been observed to undergo the Rohmer pathway, or an analogous three plus two condensation, to form **3**.[19]

Scheme 2



[U-$^{13}C_6$]Glucose, [1-$^{13}C_1$]glucose, and [1,2-$^{13}C_2$]acetate were fed to cell cultures of *Taxus chinensis*, and the major taxoid product taxuyunnanine C **(8)** was examined.[17] Acetate was found to incorporate only in the acetyl side chains of taxuyunnanine C. [U-^{13}C]Glucose was found to incorporate into the diterpenoid portion in units of two and three carbons as well as the acetyl side chains, Figure 2.

The [1-^{13}C]glucose feeding study also supports the Rohmer pathway as C(1) and C(5) of **3** were labeled as predicted. If glucose had been converted to acetate C(2), C(4), and C(5) of **3** would have been labeled. Clearly additional studies are necessary to ascertain whether two pathways exist and if so why.

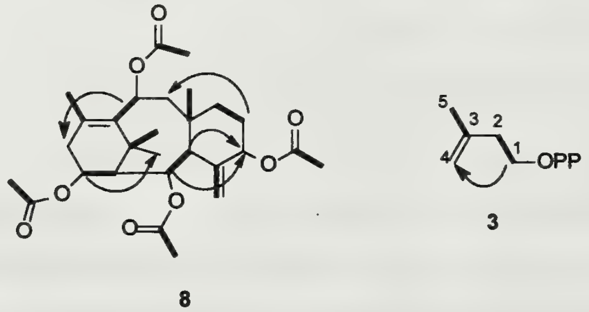
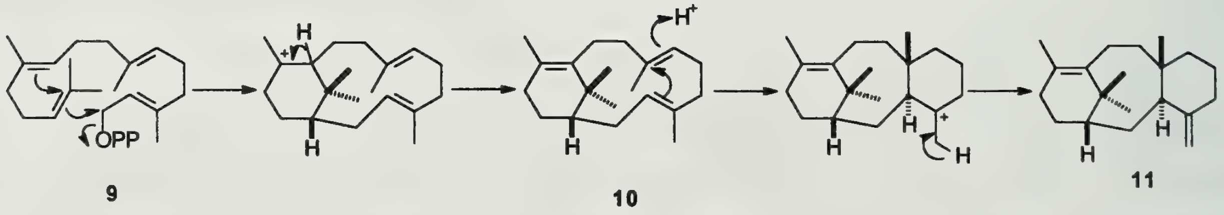


Figure 2. Incorporation of ^{13}C labels from [U-^{13}C]glucose into **8** and **3**. The bold lines and arrows represent carbon atoms that are derived from the same precursor.

Cyclization of GGPP to Taxa-4(5),11(12)-diene

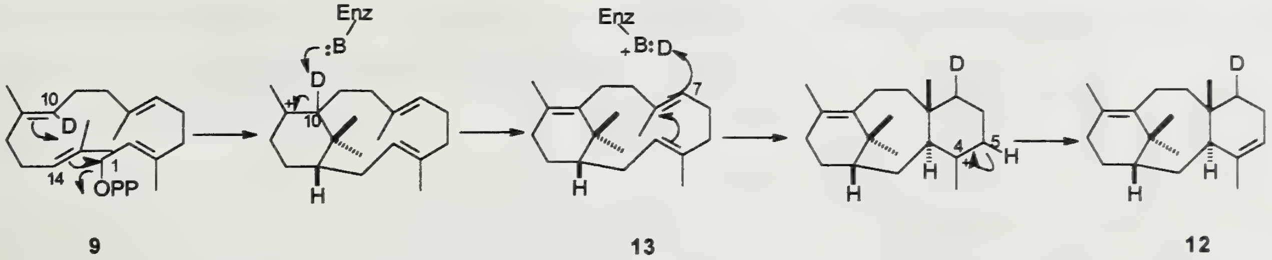
In 1987, it was proposed that GGPP **(9)** cyclized through a series of cationic rearrangements through verticillene **(10)** to form taxa-4(20),11(12)-diene **(11)**, the common precursor of the taxoids, Scheme 3.[20] In 1995, the identification of the precursor formed by *Taxus* spp. from **9** was established to be taxa-4(5),11(12)-diene **(12)** not **11**, Scheme 4.[21-24] It appears the compound remains enzyme bound until it is released as **12**. When proposed intermediates of **10** and **11** were fed to the isolated synthase enzyme, **12** was not detected.[23]

Scheme 3



Deuterium labeling studies were performed to follow proton transfers within the synthase.[23] [20-$^{2}H_3$]GGPP was converted to [20-$^{2}H_3$]**12** by taxadiene synthase with no loss of label while [4-$^{2}H_2$]**9** was converted to [5-$^{2}H_1$]**12** with loss of one deuterium. These results support the direct formation of the endocyclic double bond rather than formation of the exocyclic double bond followed by isomerization. When [10-$^{2}H_1$]**9** was fed, it was converted to [7-$^{2}H_1$]**12**. The transfer of the deuterium from C(10) of **9** to C(7) of **12** supports **9** cyclizing through an enzyme bound verticillene intermediate, Scheme 4.[23] The pyrophosphate ester leaves, forming a carbocation at C(1) which attacks C(14) of **9**. Closure of the A ring occurs by a *re*-face attack at C(10) followed by deprotonation at C(10) to yield 1*S*-verticillene **(13)** (enzyme bound). The C(10) proton that was abstracted is transferred to C(7) to initiate cyclization of the C ring, generating the carbocation at C(4). Deprotonation of C(5) then forms the endocyclic double bond yielding **12**.

Scheme 4

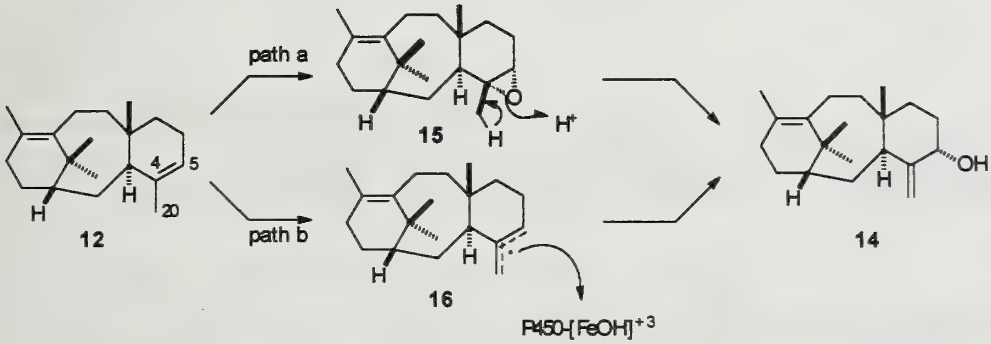


Hydroxylation of 12 to Taxa-4(20),11(12)-diene-5α-ol

The second step in the biosynthesis of paclitaxel involves the formation of taxa-4(20),11(12)-diene-5α-ol **(14)**, Scheme 5.[25] Microsomal preparations, containing cytochrome P450 enzymes, from cell cultures of *T. brevifolia* were found to catalyze the hydroxylation of **12** to **14.** The two epimers of taxa-4(20),11(12)-diene-5-ol were synthesized in the identification of the enzyme product.

Two mechanisms for the hydoxylation of **12** have been proposed, Scheme 5. The first mechanism, path a, involves the epoxidation of the 4(5) double bond **(15)**, followed by epoxide opening with deprotonation at C(20). The second mechanism, path b, involves hydrogen abstraction by cytochrome P450 at C(20) to form an allylic radical **(16)** which then binds oxygen at C(5).

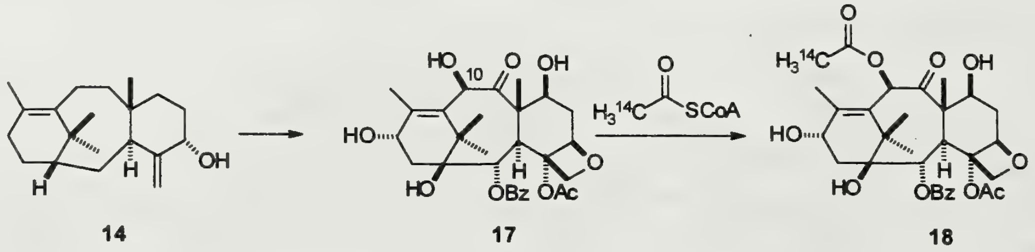
Scheme 5



Acetylation of 10-Deacetylbaccatin-III to Baccatin-III

The steps between **14** and 10-deacetylbaccatin-III **(17)** have not yet been elucidated, Scheme 6. However, the conversion of **17** to baccatin-III **(18)** has been reported.[26] Crude extracts from *Taxus baccata* roots were used for the study. It was found that when the extracts were fed **17** and [1-^{14}C]acetyl coenzyme A, labeled **18** was formed. Control experiments showed that the acetylation occurred at C(10). This enzyme is specific for the acetylation at C(10) as neither **18** nor **1** served as substrates.

Scheme 6



Biosynthesis of the Side Chain

The steps from **18** to **1** involve attachment of the side chain, *N*-benzoylphenylisoserine, **(19)** at C(13), Scheme 7. Proposed mechanisms for the biosynthesis of **19** start with phenylalanine **(20)** and proceed through *trans*-cinnamic acid **(21)**, path a, or β-phenylalanine **(22)**, path b.[27] When feeding studies with possible intermediates were conducted, it was found **19**, **20**, **22**, and phenylisoserine **(23)** were incorporated while neither **21** nor cinnamic acid epoxide **(24)** were incorporated, Table 1.[27-29] Thus, the pathway proceeds along path b.

Scheme 7

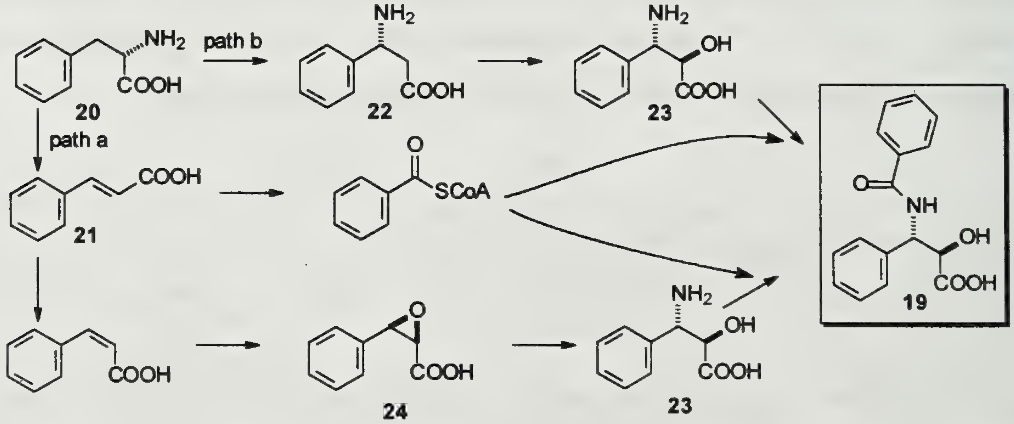
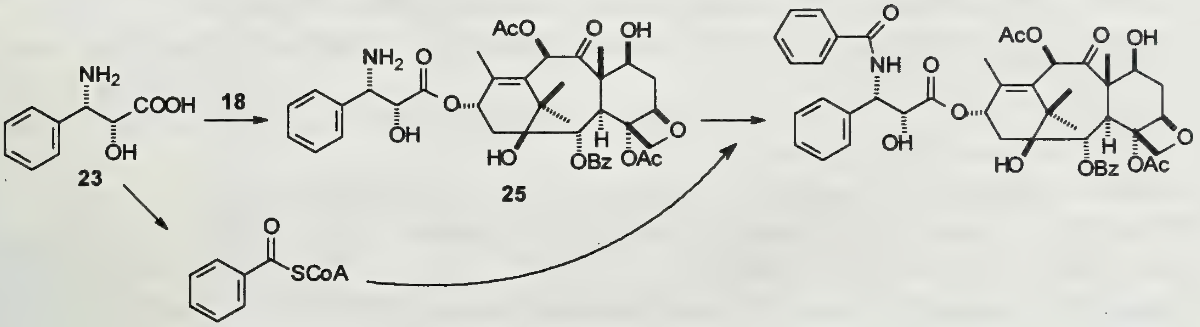


Table 1. Incorporation of precursors into paclitaxel.

Precursor	% incorporation	precursor	% incorporation
[*benzoyl*-$^2H_5$]**19**	1.7% (M+5)	[*ring*-$^2H_5$]**22**	2.8% (M+5), 2.6 % (M+10)
[*ring*-$^2H_5$]**20**	0.9% (M+5)	[*ring*-$^2H_5$]**23**	1.0% (M+5), 0.9% (M+10)
[*ring*-$^2H_5$]**21**	0% (M+5)	[*ring*-$^2H_5$]**24**	0% (M+5)

It was also observed that for **22** and **23**, a doubly labeled side chain was incorporated. This indicates that the benzoyl moiety is derived from a pathway containing **22** and **23** rather than **21**. The side chain was found to be attached in two parts. When [ring-$^2H_{10}$]**19** was fed to cambial tissue of *T. brevifolia*, no incorporation into **1** was found. However, when [*side-chain-ring*-$^2H_5$-10-*acetyl*-$^2H_3$]*N*-debenzoyltaxol **(25)** was fed, 5% incorporation into **1** was found. These results show that **23** is attached to **13** to form **25** which is then benzoylated, Scheme 8.

Scheme 8



CONCLUSION

The biosynthetic steps for **1** in *Taxus* spp. have been elucidated from IPP formation to the first oxidation to **14** and **17** to **1**. More work is necessary to clarify the IPP formation and the steps between **14** and **17**. As work progresses on the isolation and identification of the enzymes involved in the biosynthesis, genetic manipulation of cellular production of **1** will become possible, thus opening up the possibility of an abundant source of the drug for years to come.

REFERENCES

(1) Pezzuto, J. *Nat. Biotechnol.* **1996**, *14*, 1083.
(2) Stierle, A.; Strobel, G.; Stierle, D.; Grothaus, P.; Bignami, G. *J. Nat. Prod.* **1995**, *58*, 1315-1324.
(3) Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T. *J. Am. Chem. Soc.* **1971**, *93*, 2325-2327.
(4) Schiff, P. B.; Fant, J.; Horwitz, S. B. *Nature* **1979**, *277*, 665-667.
(5) Schiff, P. B.; Horwitz, S. B. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 1561-1565.
(6) Parness, J.; Kingston, D. G. I.; Powell, R. G.; Harracksingh, C.; Horwitz, S. B. *Biochem. Biophys. Res. Commun.* **1982**, *105*, 1082-1089.
(7) Nicolaou, K. C.; Yang, Z.; Liu, J. J.; Ueno, H.; Nantermet, P. G.; Guy, R. K.; Claiborne, C. F.; Renaud, J.; Coluadourous, E. A.; Paulvannan, K.; Sorensen, E. J. *Nature* **1994**, *367*, 630-634.
(8) Holton, R. A.; Somoza, C.; Kim, H.-B.; Liang, F.; Biediger, R. J.; Boatman, P. D.; Shindo, M.; Smith, C. C.; Kim, S.; Nadizadeh, H.; Suzuki, Y.; Tao, C.; Vu, P.; Tang, S.; Zhang, P.; Murthi, K. K.; Gentile, L. N.; Liu, J. H. *J. Am. Chem. Soc.* **1994**, *116*, 1597-1598.

(9) Holton, R. A.; Somoza, C.; Kim, H.-B.; Liang, F.; Biediger, R. J.; Boatman, P. D.; Shindo, M.; Smith, C. C.; Kim, S.; Nadizadeh, H.; Suzuki, Y.; Tao, C.; Vu, P.; Tang, S.; Zhang, P.; Murthi, K. K.; Gentile, L. N.; Liu, J. H. *J. Am. Chem. Soc.* **1994**, *116*, 1599-1600.

(10) Yukimune, Y.; Tabata, H.; Higashi, Y.; Hara, Y. *Nat. Biotechnol.* **1996**, *14*, 1129-1132.

(11) Strobel, G.; Stierle, A.; Stierle, D.; Hess, W. M. *Mycotaxon* **1993**, *47*, 71-80.

(12) Strobel, G.; Yang, X.; Sears, J.; Kramer, R.; Sidhu, R. S.; Hess, W. M. *Microbiology* **1996**, *142*, 435-440.

(13) Li, J.-Y.; Strobel, G.; Sidhu, R.; Hess, W. M.; Ford, E. J. *Microbiology* **1996**, *142*, 2223-2226.

(14) Stierle, A.; Strobel, G.; Stierle, D. *Science* **1993**, *260*, 214-216.

(15) Zamir, L. O.; Nedea, M. E.; Garneau, F. X. *Tetrahedron Lett.* **1992**, *33*, 5235-5236.

(16) Strobel, G. A.; Stierle, A.; van Kuijk, F. J. G. M. *Plant Sci.* **1992**, *84*, 65-74.

(17) Eisenreich, W.; Menhard, B.; Hylands, P. J.; Zenk, M. H.; Bacher, A. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 6431-6436.

(18) Rohmer, M.; Knani, M.; Simonin, P.; Sutter, B.; Sahm, H. *Biochem. J.* **1993**, *295*, 517-524.

(19) Bach, T. J. *Lipids* **1995**, *30*, 191-202.

(20) Gueritte-Voegelein, F.; Guenard, D.; Potier, P. *J. Nat. Prod.* **1987**, *50*, 9-18.

(21) Koepp, A. E.; Herazi, M.; Zajicek, J.; Vogel, B. S.; LaFever, R. E.; Lewis, N. G.; Croteau, R. *J. Biol. Chem.* **1995**, *270*, 8686-8690.

(22) Hezari, M.; Lewis, N.; Croteau, R. *Arch. Biochem. Biophys.* **1995**, *322*, 437-444.

(23) Lin, X.; Hezari, M.; Koepp, A. E.; Floss, H. G.; Croteau, R. *Biochemistry* **1996**, *35*, 2968-2977.

(24) Wildung, M. R.; Croteau, R. *J. Biol. Chem.* **1996**, *271*, 9201-9204.

(25) Hefner, J.; Rubenstein, S. M.; Ketchum, R. E. B.; Gibson, D. M.; Williams, R. M.; Croteau, R. *Chem. Bio.* **1996**, *3*, 479-489.

(26) Zocher, R.; Weckwerth, W.; Hacker, C.; Kammer, B.; Hornbogen, T.; Ewald, D. *Biochem. Biophys. Res. Commun.* **1996**, *229*, 16-20.

(27) Fleming, P. E.; Mocek, U.; Floss, H. G. *J. Am. Chem. Soc.* **1993**, *115*, 805-807.

(28) Fleming, P. E.; Knaggs, A. R.; He, X.-G.; Mocek, U.; Floss, H. G. *J. Am. Chem. Soc.* **1994**, *116*, 4137-4138.

(29) Fleming, P. E.; Floss, H. G.; Haertel, M.; Knaggs, A. R.; Lansing, A.; Mocek, U.; Walker, K. D. *Pure Appl. Chem.* **1994**, *66*, 2045-2048.

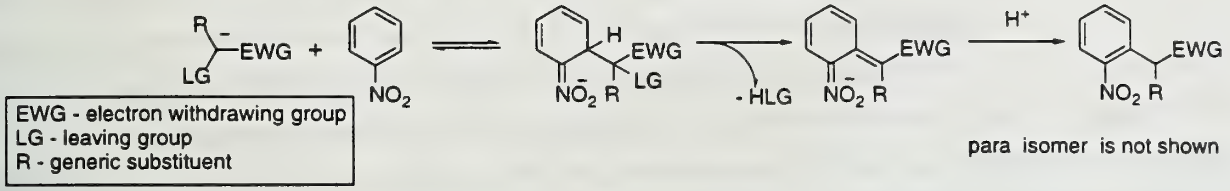
VICARIOUS NUCLEOPHILIC SUBSTITUTION

Reported by Alex Shvets March 6, 1997

INTRODUCTION

Vicarious nucleophilic substitution (VNS) is a general method to affect non-oxidative nucleophilic substitution of hydrogen in π-deficient aromatic systems.[1-4] In this transformation the incoming nucleophiles carry a nucleofuge bound to the attacking atom (Scheme 1). After the initial addition to the aromatic ring, a hydrogen atom and a leaving group undergo elimination under basic conditions. Therefore the hydrogen atom departs as a proton by a vicarious pathway. The net result is a nucleophilic substitution on the aromatic ring.

Scheme 1

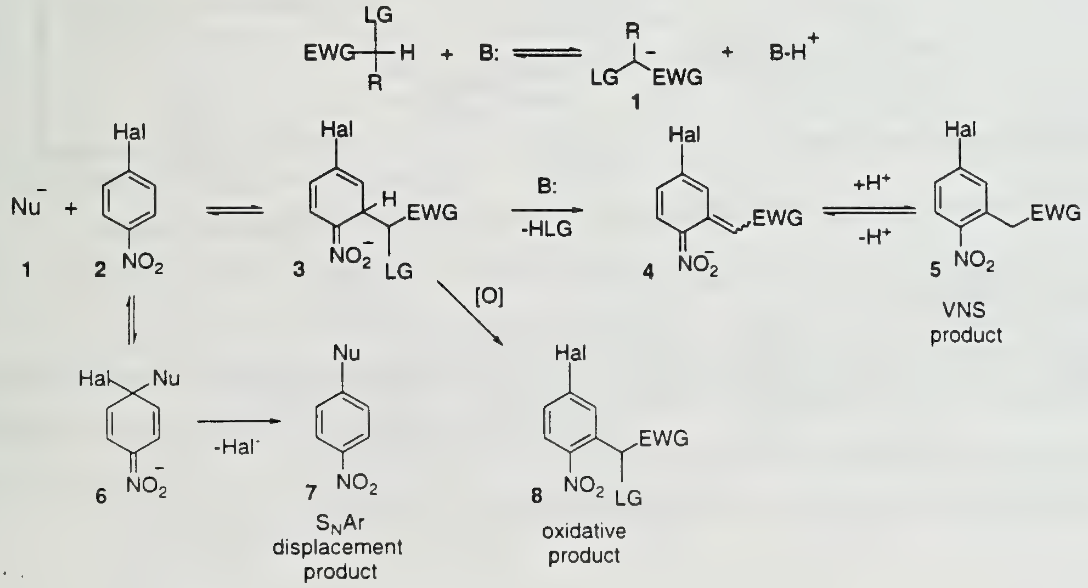


para isomer is not shown

MECHANISM

The generally accepted mechanism of VNS begins with a base-promoted formation of an anionic nucleophile (Scheme 2).[5] A reversible addition of the nucleophile **1** to a π-deficient aromatic compound **2** leads to the formation of an anionic Meisenheimer intermediate **3** which is followed by ß-elimination and the formation of a non-aromatic anionic intermediate **4**. The last step is the rearomatization upon acidic work-up leading to the VNS product **5**. Competition with the other two major pathways, aromatic nucleophilic substitution of halogens and oxidative nucleophilic substitution of hydrogen, can lead to the formation of products **7** and **8** respectively.[6,7]

Scheme 2

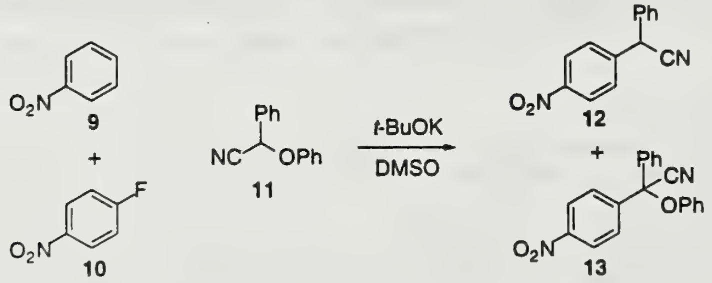


Copyright © 1997 by Alex Shvets

Kinetic data on the formation of Meisenheimer complexes indicate that many nucleophiles add faster to the carbon atom bearing hydrogen than that bearing a substituent.[8,9] The formation of a Meisenheimer complex **3** can proceed by two different pathways; a direct addition of an anionic nucleophile or through radical intermediates. Currently there is no direct evidence supporting the radical mechanism, but it is a plausible alternative.[10]

The elimination of the nucleofuge may proceed either by an E2 or E1cB mechanism. Which mechanism is operating depends on the substrate and evidence for both limiting pathways is available.[2] For example, when 4-D-nitrobenzene is combined with chloromethyl phenyl sulphone in competition with nitrobenzene only the secondary kinetic isotope effect is observed $k_H/k_D = 0.90 \pm 0.04$.[5] The absence of a primary isotope effect can be rationalized by assuming that the rate determining step is a formation of the Meisenheimer complex rather than a transfer of hydrogen in the ß-elimination step. The secondary kinetic isotope effect data correlates well with what is usually observed for rehybridization of a carbon atom from sp^2 to sp^3.[11] For 1-D-2,4-dinitrobezene reacting with 1-chloropropyl and 1-chlorobenzyl phenyl sulfones, values of $k_H/k_D = 2.9$ and $k_H/k_D = 6.5$ respectively were found.[2] The observed primary kinetic isotope effect suggests that in these cases hydrogen is abstracted in the rate determining step.

Reaction of nitrobenzene **9** and 4-fluoronitrobenzene **10** with phenoxyphenylacetonitrile **11** under basic conditions exhibited intermolecular competition between substitution of fluorine and hydrogen.[5] Compound **13** dominated at low base concentration while the VNS product **12** was observed as the major product when an excess of base was used. Sulfenamides and hydroperoxides, used in amination and hydroxylation versions of the VNS reaction, demonstrated the same dependence of reactivity on base concentration in the competition reaction.[12,13] These data suggest that the elimination process leading to the VNS product can effectively compete with the nucleophilic substitution of aromatic halogens.



t-BuOK/11 ratio	yield, % 13	12
1.0	54	10
2.0	36	30
4.0	trace	50

Figure 1. Effect of increasing base concentration on an intermolecular competition reaction.

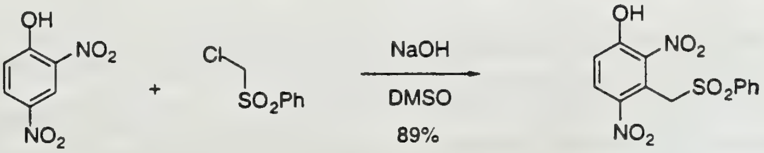
GENERAL REACTIVITY

A feature common to all VNS reactions is the requirement that at least two equivalents of base be used for deprotonation of the nucleophile and the resulting σ-adduct.[2] Single alkylation is observed exclusively because the intermediate **4** is anionic and, therefore, unreactive toward addition of a second nucleophile. Multiple alkylation can only be achieved in polynitrated benzene derivatives.[14]

Functional Group Compatibility

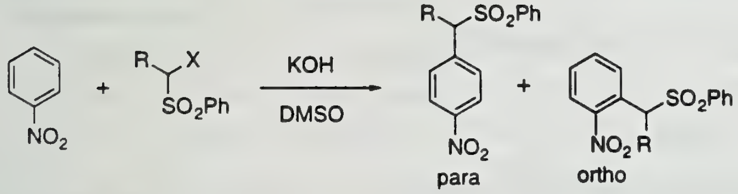
The reactive electrophile can possess different substituents on the aromatic ring. Electron withdrawing groups (trifluoromethyl, cyano, alkyl sulfoxides) and some electron donating groups (alkylamines, ethers, thio ethers) are compatible with the reaction conditions.[15,16] However, yields in the later case may be lower. Carboxylic acids and halogens, with the exception of fluorine in some cases, do not interfere with the normal course of the reaction. Phenolic groups are not compatible because they are ionized under basic reaction conditions. However, this obstacle can be overcome by having two nitro groups on the ring (Scheme 3).[14] Substituted benzaldehydes often react to give epoxides by the competing Darzens condensation.[2]

Scheme 3



Control of Regioselectivity

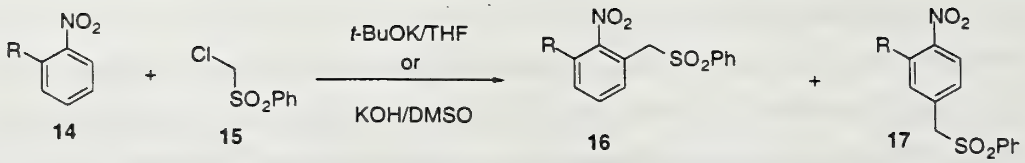
The dependence of ortho/para regioselectivity on the structure of the carbanions is illustrated by the reaction of nitrobenzene with a series of different derivatives of methyl phenyl sulfone (Figure 2).[16] Tertiary carbanions tend to react at the para-position and secondary carbanions usually give a mixture of both ortho and para products. The bias for substitution at the para-position increases as one proceeds down Group 17 of the periodic table. 1,3-Dinitrobenzene prefers to react at the less hindered 4-position.[16]



X	R	total yield, %	ratio of p:o
F	H	63	1:3
Cl	H	75	1:1
Br	H	40	2:1
I	H	20	6:1
Cl	Et	68	1:0
Cl	Ph	93	1:0
Cl	Cl	35	1:0

Figure 2. Orientation of VNS in nitrobenzene.

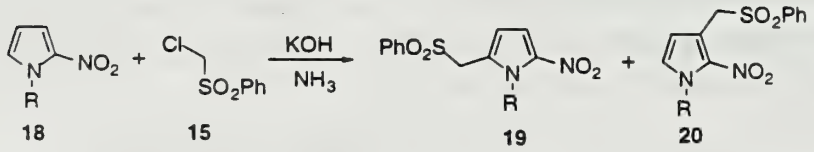
By changing the reaction conditions from sodium or potassium hydroxide in dimethyl sulfoxide to potassium *tert*-butoxide in tetrahydrofuran it was possible to achieve higher selectivity for ortho-substitution.[17] This particular preference is observed for many secondary carbanions. Addition of 18-crown-6 to the reaction mixture in THF eliminates this effect.



R	*t*-BuOK/THF			KOH/DMSO		
	yield, %	para (17)	ortho (16)	yield, %	para (17)	ortho (16)
H	61	0	100	82	58	42
2-NMe$_2$	32	0	100	63	84	16
2-OMe	56	7	93	75	96	4
2-CF$_3$	93	trace	100	87	44	56
2-Cl	99	0	100	77	85	15

Figure 3. Solvent effect in the VNS reaction of nitroareanes with chloromethyl phenyl sulfone.

In the series of nitro substituted 5-membered ring heterocycles, the following regioselectivity is observed.[18] 2-Nitrothiophene prefers substitution at the 3-position, even with tertiary carbanions which tend to react with nitrobenzene at the para-position. 2-Nitrofuran prefers to react at the 5-position. Results with *N*-protected 2-nitropyrroles indicate that mainly electronic factors determine this difference in reactivity. For example, 1-propyl-2-nitropyrrole reacts with chloromethyl phenyl sulfone in a similar fashion to 2-nitrofuran giving the product of VNS at the 5-position **19**. On the contrary, 1-(4-toluenesulfonyl)-2-nitropyrrole gives only substitution at the 3-position **20** just as 2-nitrothiophene.

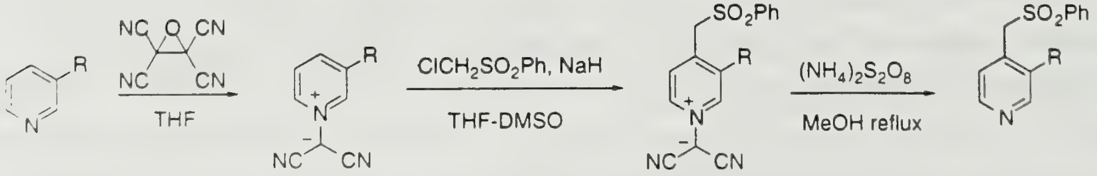


R	ratio **19**:**20**	yield, %
n-Pr	1:0	93
i-Pr	1:0	68
SO$_2$-Tol	0:1	83
CH$_2$OCH$_3$	1:5	88

Figure 4. Reactions of nitropyrrole derivatives with chloromethyl sulfone.

Types of Electrophiles Participating in VNS

Unactivated arenes generally do not participate in VNS reactions. The presence of one nitro group is usually sufficient for the reaction to take place. π-Deficient heterocycles, such as pyridines and pyridazines can be activated through their dicyanomethylide derivatives (Figure 5).[19,20] However, there are some exceptions; azulene, and some heterocycles, such as 1,2,4-triazines and pteridines, do not require additional activation to react in VNS.[21,22]



R	overall yield, %
Et	76
OEt	75

Figure 5. VNS of pyridines via their dicyanomethylide derivatives.

Types of Nucleophiles Participating in VNS

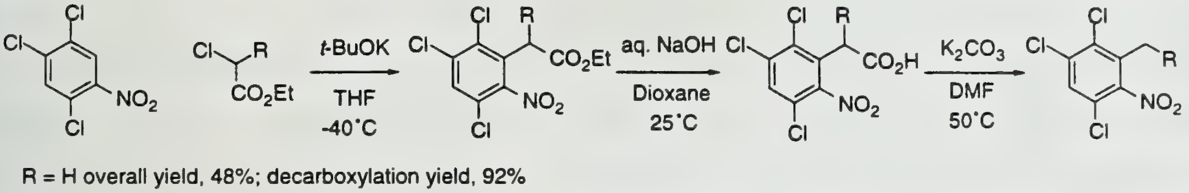
Vicarious nucleophilic substitution is a general process in which different nucleophiles bearing a variety of leaving groups can be used.[2-4] Many carbon acids such as alkyl esters, alkyl diesters, alkyl phosphine oxides, nitro alkyl, cyano alkyl, alkyl sulfonyl derivatives carrying a nucleofuge at the anionic center can be employed in the reaction. Common leaving groups are halogens, phenols, thiophenols, benzotriazole, and cyanide.

SYNTHETIC UTILITY

Alkylation

Different alkyl chloroacetates can be used to carry out alkylation under VNS conditions.[23-26] Ester intermediates initially formed can be further hydrolyzed and decarboxylated providing alkyl nitroarenes (Scheme 4).[23] Modest yields of alkyl nitroarenes can also be achieved by direct alkylation with butyl trifluoromethyl sulfone.[26] The trifluoromethylsulfonyl moiety provides efficient stabilization for the carbanion and is a relatively good leaving group.

Scheme 4



It is possible to achieve isocyanomethylation of 1-nitronaphthalene when phenylthiomethyl isocyanide is used as the nucleophile (Figure 6).[27] Thiophenol was eliminated in preference over isocyanate and no phenyl ether products were observed in all the reactions studied.

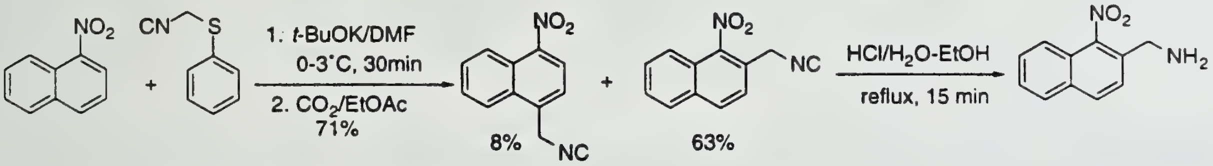
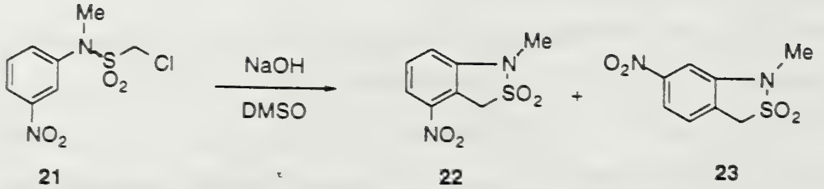


Figure 6. Isocyanomethylation of 1-nitronaphthalene.

Ring Formation by the VNS Method

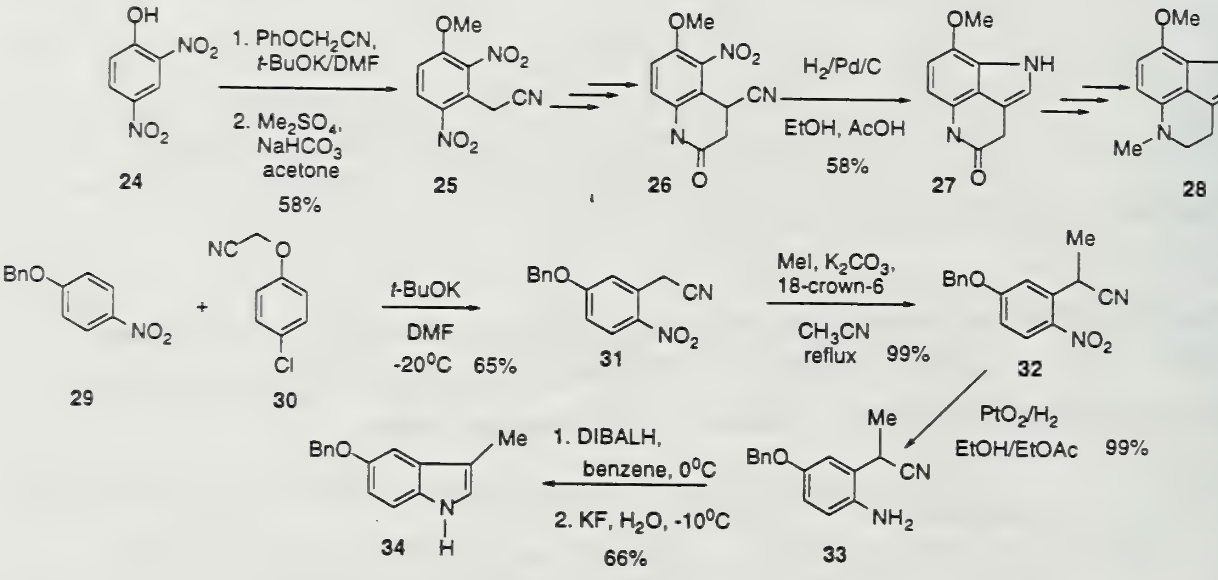
The intramolecular version of VNS reactions[28-34] allows the construction of 5 and 6-membered ring sultams (Scheme 5).[28] Compound **21** reacts under standard VNS conditions affording **22** and **23** in a 4:1 ratio. The ortho-closure, with respect to the nitro group, dominates in this reaction. When fluorine is present in the starting material at the 4-position, S_NAr is a major competing process. This competition favors the closure at the para-position.

Scheme 5



A key VNS step is featured in the synthesis of precursors of a series of indole alkaloids (Scheme 6).[30,31] The synthesis of alkaloid **28** uses this reaction for the conversion of **24** to **25**. In the synthesis of the protected indole **34**, this method is used early for the introduction of a cyano methyl group into compound **29**. The common feature of these syntheses is the ability to reductively cyclize nitro/amine groups and nitrile to form a pyrrole ring. The conversions of **26** to **27** and **33** to **34** correspond to these transformations. These functional groups were initially placed in ortho relationship by the VNS reaction.

Scheme 6



Vicarious nucleophilic substitution is not limited to mononuclear activated aromatics and was applied for the synthesis of more complex molecules as well. The key step in the synthesis of aklavinone was a one pot Michael addition followed by intramolecular VNS ring closure.[35]

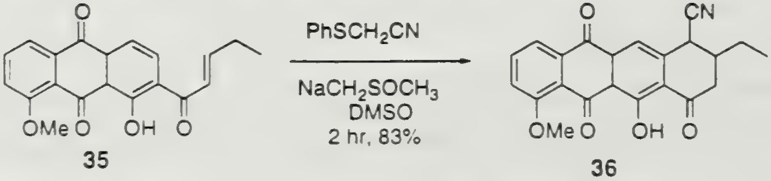
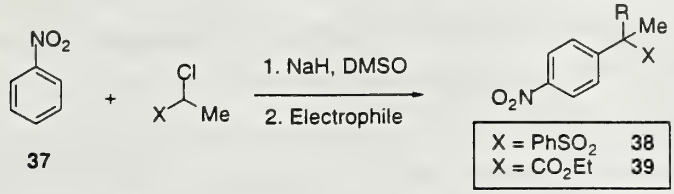


Figure 7. Tandem Michael/VNS ring closure.

Functionalization of the α-Carbon

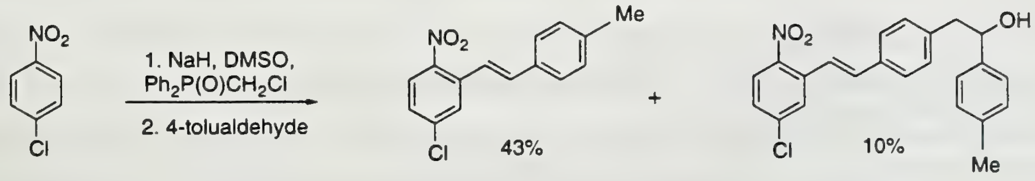
Recently it was reported that the intermediate anion **4** (Scheme 2) can be trapped with alkyl halides as electrophiles.[36] Thus, one pot VNS/alkylation method provides a convenient route for the construction of quaternary centers. An earlier report on consecutive VNS/Horner-Wittig reaction, leading to the selective synthesis of *E*-stilbenes, is mechanistically similar (Scheme 7).[37]



Electrophile	yield, %	
	38	39
MeI	74	60
$CH_2{=}CHCH_2Br$	80	67
$PhCH_2Br$	72	63

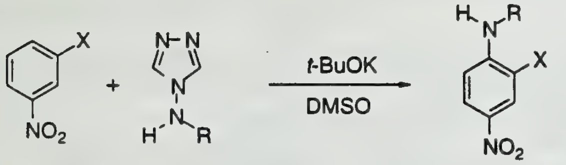
Figure 8. Consecutive VNS/alkylation reactions.

Scheme 7



Amination and Hydroxylation

Vicarious nucleophilic substitution can also be used to form carbon-nitrogen and carbon-oxygen bonds. Use of 4-amino-1,2,4-triazoles in the exclusive formation of para-substituted amino nitrobenzenes has been documented (Figure 9).[38] The advantage of this method is the ability to introduce *N*-alkylamines. Alternative reagents for amination of substituted nitrobenzenes are sulfenamides, such as *N,N*-tetramethylenethiocarbamoyl and 2,4,6-trichlorophenyl. For example, benzothiazolesulfenamide under a different set of reaction conditions gave regioselective amination of 1-nitrophenol at the positions 2 and 4.[13]

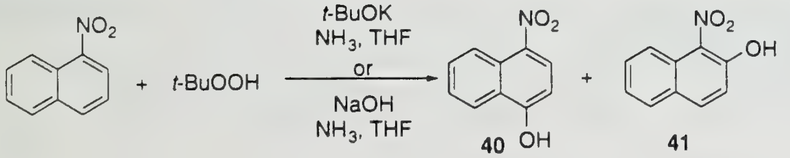


X	R	yield, %
H	CH_3	79
H	C_2H_5	81
H	CH_2Ph	17
Cl	C_2H_5	82
CO_2H	CH_3	98

Figure 9. Amination of meta substituted nitrobenzenes with *N*-substituted 4-amino-1,2,4-triazoles.

To carry out hydroxylation under VNS conditions, *tert*-butyl and cumyl hydroperoxides can be employed.[12] Good regioselectivity was achieved for 1-nitronaphthalene by varying the nature of base used in the reaction (Scheme 8). Regioselectivity for simple nitrobenzenes cannot be easily controlled.

Scheme 8



product	t-BuOK yield %	NaOH yield %
40	87	trace
41	trace	89

CONCLUSION

Reaction sequences based on VNS are finding numerous applications. The power and the synthetic value of the method are in the ability to use variety of electrophiles as well as nucleophiles. Mild conditions and moderate control over regioselectivity by tuning the reaction conditions are useful features of the method. The method allows the formation of carbon-carbon, carbon-nitrogen and carbon-oxygen bonds. The main disadvantage of the method is a competing oxidative nucleophilic substitution process. The nature of the method requires the presence of an electron withdrawing group at the α-position of the nucleophile. The removal of this group may pose a problem in some syntheses.

REFERENCES

(1) Makosza, M. *Chimia* **1994**, *48*, 499-500.
(2) Makosza, M.; Winiarski, J. *Acc. Chem. Res.* **1987**, *20*, 282-287.
(3) Makosza, M. *Synthesis* **1991**, 103-111.
(4) Makosza, M. *Russian Chem. Bull.* **1996**, *45*, 491-504.
(5) Makosza, M.; Glinka, T. *J. Org. Chem.* **1983**, *48*, 3860-3861.
(6) Makosza, M.; Sypniewski, M. *Tetrahedron* **1994**, *50*, 4913-4920.
(7) Makosza, M.; Sypniewski, M.; Glinka, T. *Tetrahedron* **1996**, *52*, 3189-3194.
(8) Terrier, F. *Chem. Rev.* **1995**, *95*, 2261-2280.
(9) Terrier, F. *Chem. Rev.* **1982**, *82*, 77-152.
(10) Bunnett, J. F. *Acc. Chem. Res.* **1978**, *11*, 413-420.
(11) Strausz, O. P.; Safarik, I.; Gunning, H. E. *J. Am. Chem. Soc.* **1972**, *94*, 1828
(12) Makosza, M.; Sienkiewicz, K. *J. Org. Chem.* **1990**, *55*, 4979.
(13) Makosza, M.; Bialecki, M. *J. Org. Chem.* **1992**, *57*, 4784-4785.
(14) Makosza, M.; Ludwiczak, S. *J. Org. Chem.* **1984**, *49*, 4562-4563.
(15) Makosza, M.; Winiarski, J. *J. Org. Chem.* **1984**, *49*, 1494-1499.
(16) Makosza, M.; Golinski, J. *J. Org. Chem.* **1984**, *49*, 1488-1494.
(17) Makosza, M.; Glinka, T.; Kinowski, A. *Tetrahedron* **1984**, *40*, 1863-1868.
(18) Makosza, M.; Kwast, E. *Tetrahedron* **1995**, *51*, 8339-8354.
(19) Itoh, T.; Matsuya, Y.; Nagata, K.; Okada, M.; Ohsawa, A. *J. Chem. Soc., Chem. Commun.* **1995**, 2067.
(20) Itoh, T.; Nagata, K.; Okada, M.; Ohsawa, A. *Chem. Pharm. Bull.* **1993**, *41*, 220-222.
(21) Makosza, M.; Kuciak, R., Wojciechowski, K. *Liebigs Ann. Chem.* **1994**, 615-618.
(22) Rykowski, A.; Makosza, M. *Tetrahedron Lett.* **1984**, *25*, 4795-4796.
(23) Bull, D. J.; Fray, J.; MacKenny, M. C.; Malloy, K. A. *Synlett* **1996**, 647-648.
(24) Stahly, P. G.; Stahly, B. C.; Lilje, K. C. *J. Org. Chem.* **1984**, *49*, 579-580.
(25) DeBoos, G. A.; Milner, D. J. *Synth. Commun.* **1994**, *24*, 965-976.
(26) Wrobel, Z.; Makosza, M. *Org. Prepr. Procedures Int.* **1990**, *22*, 575-578.
(27) Makosza, M.; Kinowski, A.; Ostrowski, S. *Synthesis* **1993**, 1215-1216.
(28) Makosza, M.; Wojciechowski, K. *Tetrahedron Lett.* **1984**, *25*, 4791-4792.
(29) Tyrala, A.; Makosza, M. *Synthesis* **1993**, 1215-1216.
(30) Marino, J., P.; Hurt, C., R. *Synth. Commun.* **1994**, *24*, 839-848.
(31) Makosza, M.; Stalewski, J. *Tetrahedron* **1995**, *51*, 7263-7276.
(32) Ostrowski, S. *Heterocycles* **1996**, *43*, 389-396.
(33) Makosza, M.; Hoser, H. *Heterocycles* **1994**, *37*, 1701-1704.
(34) Robinson, R. P.; Donahue, K. M.; Son, P. S.; Wagy, S. D. *J. Heterocycl. Chem.* **1996**, 33, 287-293.
(35) Murphy, R., A.; Cava, M., P. *Tetrahedron Lett.* **1984**, *25*, 803-806.
(36) Lawrence, N., J.; Liddle, J. *Synlett* **1996**, 55-56.
(37) Lawrence, N. J.; Liddle, J.; Jackson, D. A. *Tetrahedron Lett.* **1995**, *36*, 8477-8480.
(38) Katritzky, A., R.; Laurenzo, K., S. *J. Org. Chem.* **1988**, *53*, 3978-3982.

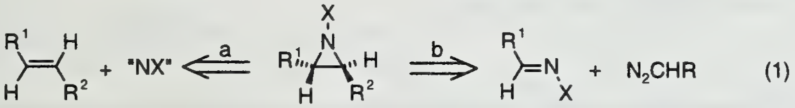
SYNTHETIC METHODS FOR CATALYTIC ASYMMETRIC AZIRIDINATION

Reported by Michael S. Wendland March 27, 1997

INTRODUCTION

Aziridines are versatile precursors in synthetic organic chemistry. In the ever-growing field of asymmetric synthesis, chiral aziridines play an important role as auxiliaries, ligands for transition metals and substrates for the preparation of biologically active compounds.[1] A variety of routes to access chiral, nonracemic aziridines exist;[1] however, most of them rely on multi-step transformations of enantiomerically pure starting materials. Another synthetic route to chiral, nonracemic aziridines makes use of asymmetric transformations of carbon-carbon or carbon-nitrogen double bonds. These pathways are particularly attractive because they are amenable to enantioselective catalysis.

Two general methods have been developed for catalytic asymmetric aziridination (CAA): asymmetric catalytic transfer of nitrenes to alkenes (Eq. (1), path a) and asymmetric catalytic transfer of carbenoids to imines (Eq. (1), path b). Herein are reported the most recent advances in the area of these two promising methods of CAA.



(1)

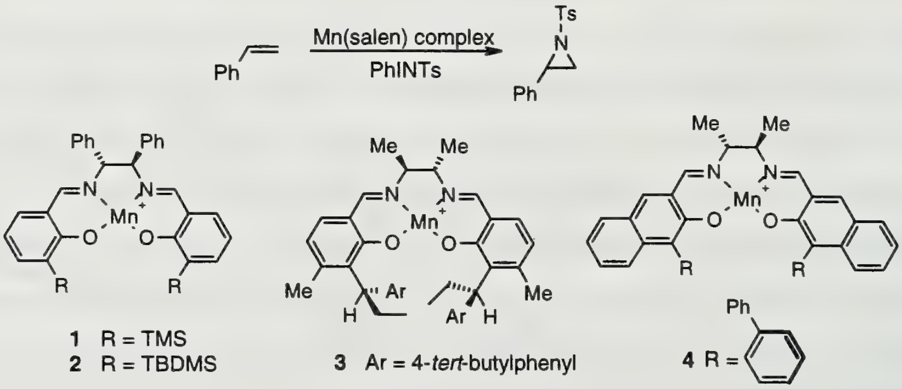
NITRENE TRANSFER TO ALKENES

Nitrene transfers to alkenes can be metal catalyzed and are believed to be mediated by catalytic formation of reactive metal-nitrenoid intermediates. Simple iron and manganese porphyrins were observed to catalyze the transfer of nitrenes to alkenes.[2] For example, derivatives of tetraphenylporphyrins (TPP) catalyze the aziridination of olefins with [*N*-(4-toluenesulfonyl)imino] phenyliodinane (PhINTs) as a nitrene source. The lack of an asymmetric environment in these porphyrin systems led to the formation of racemic products. Observations from infrared and proton NMR spectroscopy suggested that a metal-nitrenoid intermediate was formed[3] which was responsible for the nitrene transfer to the alkene.[4] Metal porphyrins are not very amenable to serve as asymmetric catalysts because the metal center is far removed from the periphery where the attachment of chiral moieties could be possible.

In attempts to bring the chiral environment closer to the metal, manganese salen complexes

Copyright © by Michael Wendland

(Figure 1) have been synthesized and studied for their ability to catalyze the asymmetric aziridination of alkenes with PhINTs. Silyl-substituted manganese salen complexes **1** showed no enantioselectivity for the reaction of PhINTs with styrene.[5] Further modification of the ligand substituents led to Mn(salen) complexes **3** and **4**. Complex **3** was found to give moderate selectivity (61% ee, *R*) for the aziridination of styrene, albeit in very low yield (9%).[6] Complex **4** catalyzed formation of the aziridine of styrene in moderate yield (76%) and very good enantioselectivity (94% ee, *S*).[7] Modification of the substrate from styrene to substituted styrene (4-Cl and 4-Me) did not lead to any significant changes in the yields or enantioselectivities.



1 R = TMS
2 R = TBDMS

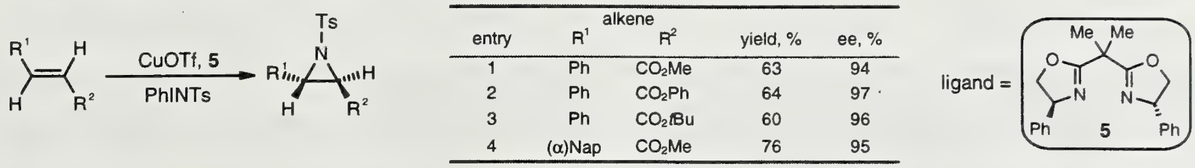
3 Ar = 4-*tert*-butylphenyl

4 R =

Figure 1. Manganese salen complexes for CAA.

Another series of catalysts for aziridine formation by nitrene transfer is based on copper salts. It is already 30 years since copper powder was found to catalyze the decompostion of benzenesufonyl azide, and in the presence of cyclohexene form the aziridine.[8] Initially, copper (I) and (II) salts were studied without chiral ligands to probe the effects of solvent, counterion and temperature.[9] In addition, the nitrene source (of which only PhINTs has been reported to be efficient for nitrene transfer) and reaction scope were studied.[10] An advantage over copper salts over other metal catalysts is that no insertion products are formed in the aziridination of alkyl-substituted olefins. Furthermore, as with porphyrin catalysts, the aziridination of cis-alkyl-substituted alkenes produced only cis-aziridines, and cis-aryl-substituted alkenes produced mixtures of cis and trans aziridines. This implies a stepwise mechanism for nitrene addition to aryl-substituted alkenes.

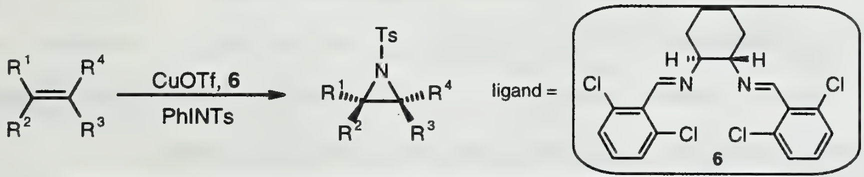
Creating a chiral environment around copper led recently to some interesting catalysts. Various derivatized bis(oxazolines),[11] pyrazoles[12] and axially dissymmetric diamine[13] ligands have been shown to react with styrene to produce the tosyl aziridine in low yield with low enantioselectivities. Figure 2 illustrates some excellent results obtained for the CAA of trans-cinnamate esters using the chiral ligand **5** and CuOTf.[14] It was noted that the steric bulk of the olefin substituents did not significantly affect the yield or ee; however, modification of the ligand and the use of a different solvent were needed to optimize the CAA of styrene to an 89% yield and 63% ee.



entry	alkene R^1	alkene R^2	yield, %	ee, %
1	Ph	CO_2Me	63	94
2	Ph	CO_2Ph	64	97
3	Ph	CO_2tBu	60	96
4	(α)Nap	CO_2Me	76	95

Figure 2. Enantioselective aziridination of trans-cinnamate esters with PhINTs catalyzed by **5** / CuOTf.

Figure 3 highlights some results of using a conformationally restricted bis(imine) ligand **6** and CuOTf for the aziridination of various alkenes.[15] It was again observed that the reaction of cis-aryl-substituted alkenes produced a mixture of cis and trans aziridines implying a stepwise addition. Ligands with a 1,2-diphenyldiaminoethane bridge instead of the cyclohexyl bridge showed very low enantioselectivity presumably due to conformational mobility. Comparison of ligands with different substitution patterns on the aromatic rings showed the enantioselectivity was also dependent on the electronic nature of the ligand.



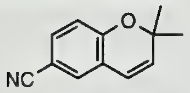
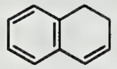
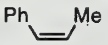
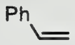
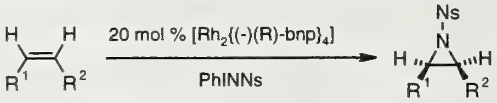
entry	alkene	yield, %	ee,%	configuration
1		75	>98	(3*R*, 4*R*)
2		70	87	(1*R*, 2*S*)
3		79 (cis : trans, 3:1)	67 (cis) 81 (trans)	(1*R*, 2*S*) (1*S*, 2*S*)
4		79	66	(*R*)

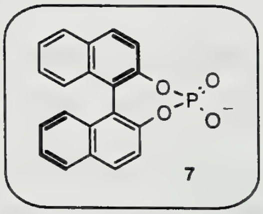
Figure 3. Enantioselective aziridination of alkenes with PhINTs catalyzed by **6** / CuOTf.

In related examples, rhodium (II) salts, such as rhodium tetraacetate [$Rh_2(OAc)_4$], were also found to have reasonable catalytic ability for CAA.[16] Attempts to make chiral rhodium catalysts competitive with those of Cu catalysts have not been successful to date. Figure 4 shows that the best results observed used a chiral 1,1'-binapthyl-2,2'-diylphosphate ligand **7** with $Rh_2(OAc)_4$ which generated ([Rh_2{(*R*)-(-)-bnp}$_4$]) as a chiral catalyst. The use of [*N*-(*p*-nitrobenzenesulfonyl)imino] phenyliodinane (PhINNs) as the nitrene source showed modest enantioselectivity with this catalyst in the CAA of styrene and *cis*-β-methylstyrene.[17]



alkene			
R^1	R^2	yield, %	ee, % (cis)
Ph	H	75	55
Ph	Me	75	73

bnp =



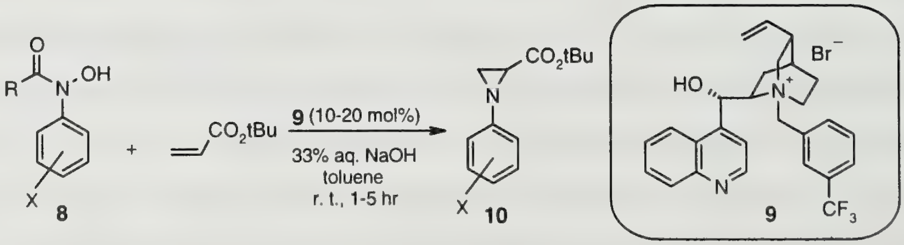
7

Figure 4. CAA of styrene and *cis*-β-methyl styrene with PhINNs catalyzed by **7** / $Rh_2(OAc)_4$.

A mechanism for the metal-catalyzed transfer of a nitrene to an alkene has been proposed. Due to the erosion in selectivity observed for aryl-substituted alkenes as opposed to alkyl-substituted alkenes, a stepwise mechanism was favored over a concerted one.[18,19] However, lack of a significant difference in rate for the aziridinations of styrene and 4-nitrostyrene with PhINTs catalyzed by $CuClO_4$ in dichloromethane and acetonitrile suggests that a charged intermediate is not involved.[9] A radical trap experiment for the aziridination of alkyl-substituted olefins supports a concerted additon.[9] These results led to the proposal of a mechanism for the radical stepwise addition of a nitrene to an olefin from a metal-nitrenoid reactive intermediate for aryl-substituted olefins and a concerted addition for alkyl-substituted olefins. It is still debated; nonetheless, that the addition is stepwise regardless of the olefin substituents. No conclusive evidence has been reported to settle this debate.

Recently a novel, non-metal catalyzed CAA by nitrene transfer to alkenes was reported. The quaternary salt **9** derived from a *Cinchona* quinine alkoloid was used as a phase-transfer catalyst for the enantioselective aziridination of electron deficient alkenes by hydroxamic acids **8** (Scheme 1).[20] This process was found to be extremely sensitive to the aromatic substituent X, and in fact, no aziridine formed when X = 4-nitro. The aziridine **10** was produced in 61% yield with an ee of 50% when R = *t*-Bu and X = H.

Scheme 1

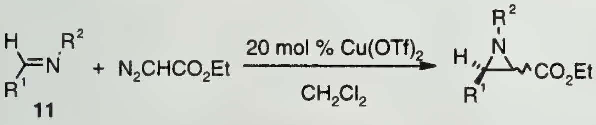


CARBENOID TRANSFER TO IMINES

Diazocarbonyl compounds have been found to be convenient carbenoid precursors. The synthetic accessibility of imines and diazocarbonyls, and the lack of organic byproducts make the CAA of imines with diazocarbonyl compounds an exciting prospect. Another advantage lies in the prospects of synthetic convergence with coupling two complex fragments in a controlled fashion.

There have been many attempts to use transition metals to catalyze the transfer of carbenoid species to imines, but few succeeded to do so in an asymmetric fashion. Recently, methylrhenium trioxide (MTO) was shown to efficiently catalyze the aziridination of a wide variety of arylimines.[21] However, there are no reports yet of MTO being used for CAA.

The Cu catalysts which gave excellent ee for the transfer of a nitrene to alkenes (discussed above) have also been studied for the transfer of carbenoids to imines. Figure 5 shows that the reaction of arylimines **11** with ethyl diazoacetate and a catalytic amount of $Cu(OTf)_2$ provided the corresponding aziridines with modest diastereospecificity and in high yield.[22] Diastereospecificities better than 20:1 were achieved by increasing the steric bulk of the imine nitrogen group; however, the yield was dramatically reduced (35%).

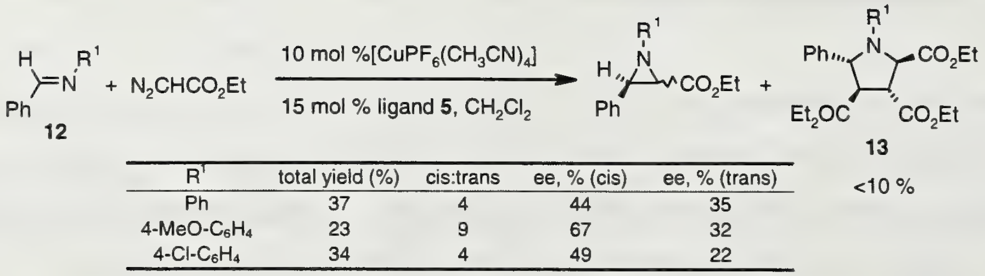


entry	R^1	R^2	temp., °C	cis:trans	total yield,%
1	Ph	Ph	-25	1.7:1	80
2	*t*-Bu	Ph	-25	0.6:1	90
3	H	Ph	0	-	85
4	Ph	$SiMe_3$	-25	>20:1	35

Figure 5. Aziridination of arylimines with ethyl diazoacetate catalyzed by $Cu(OTf)_2$.

The CAA of benzaldimines with ethyl diazoacetate catalyzed by copper and chiral ligand **5** produced the resultant aziridines in low yield with modest diastereospecificity and ee, Figure 6.[23] For all reactions, racemic pyrrolidine **13** was observed as a minor product. A similar study was done using $Rh_2(OAc)_4$ as a catalyst with various arylimines and ethyl diazoacetate, but yields and diastereospecificity were low and only racemic products were obtained.[24]

The proposed mechanistic rational for the transfer of carbenoids to imines involves an equilibrium between a complexed intermediate **14** and an uncomplexed ylide **15**, Scheme 2.[23] The complexed ylide forms enantiomerically pure aziridine, whereas the free ylide forms a racemic aziridine. Racemic pyrrolidine is formed from the uncomplexed ylide if diethyl maleate or fumarate is present. The equilibrium between complexed ylide and free ylide then determines the enantioselectivity of the entire process.

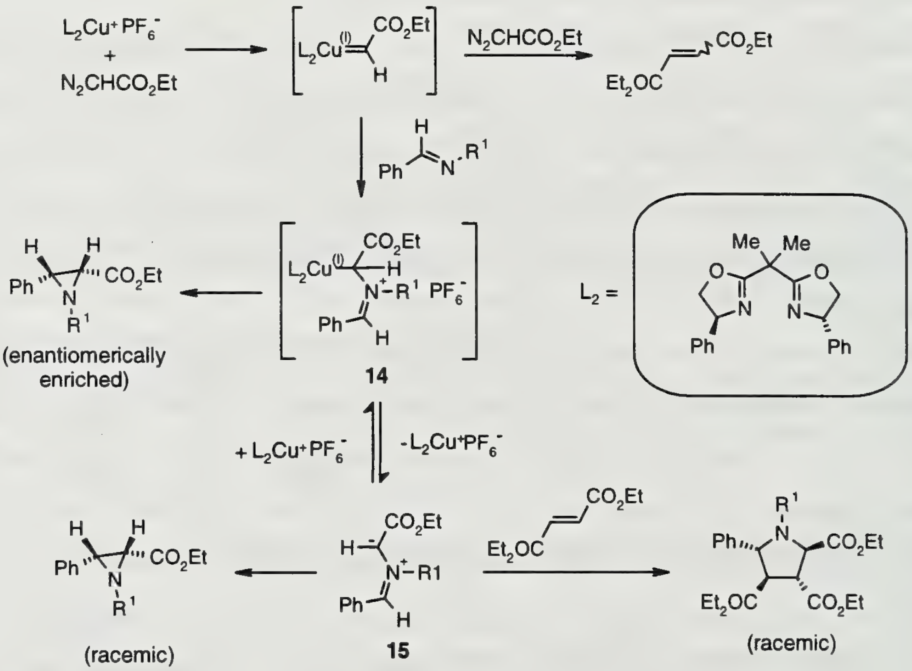


12
10 mol %[CuPF$_6$(CH$_3$CN)$_4$]
15 mol % ligand 5, CH$_2$Cl$_2$
13
<10 %

R^1	total yield (%)	cis:trans	ee, % (cis)	ee, % (trans)
Ph	37	4	44	35
4-MeO-C$_6$H$_4$	23	9	67	32
4-Cl-C$_6$H$_4$	34	4	49	22

Figure 6. CAA of several benzaldimines with ethyl diazoacetate catalyzed by **5** / CuPF$_6$.

Scheme 2



The most exciting results have come from a study in which CAA is mediated by sulfur ylides.[25] The proposed catalytic cycle in which metal-carbenoid formation regulates the formation of sulfur ylide is illustrated in Figure 7. An electron withdrawing group was placed on the nitrogen to activate the nitrogen for nucleophilic attack by the sulfur ylide, and it was noted that silyl and phenyl groups were not sufficiently activating. *N*-Tosylbenzaldimines were found to produce aziridines through carbenoid transfer in excellent yields (>90%) with modest diastereospecificity. Due to the difficulty in removing the tosyl protecting group; however, other nitrogen protective groups that were easier to remove were tried. One of these, *N*-[β-(trimethylsilyl)ethanesulfonyl] (SES) was found to be the most useful for this class of imines. Figure 8 shows the model reaction with chiral sulfide **16**. The use of stoiciometric amounts of Me$_2$S and catalytic amounts of Cu(acac)$_2$ instead of Rh$_2$(OAc)$_4$ improved the yield of the carbenoid transfer. The observation that no aziridine forms in the absence of a sulfide suggests that some other reactive species may be involved, such as sulfur ylides.

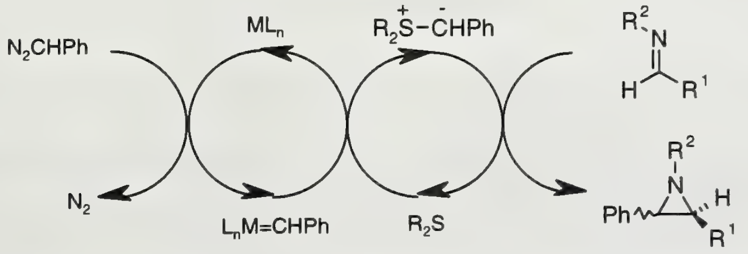
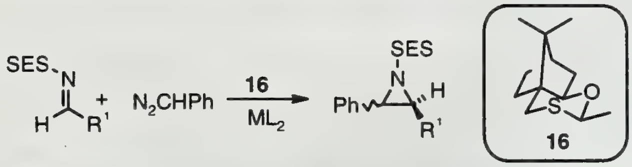


Figure 7. CAA mediated by sulfur ylide.



entry	R^1	equiv. **11**	ML_2	yield, %	trans:cis	ee, % (config.)
1	Ph	1.0	$Rh_2(OAc)_4$	55	3:1	97 (*R*,*R*)
2	Ph	1.0	$Cu(acac)_2$	83	3:1	95 (*R*,*R*)
3	Ph	0.2	$Cu(acac)_2$	62	3:1	90 (*R*,*R*)
4	$4\text{-}MeC_6H_4$	1.0	$Rh_2(OAc)_4$	88	3:1	95 (*R*,*R*)
5	$4\text{-}MeC_6H_4$	0.2	$Cu(acac)_2$	50	3:1	88 (*R*,*R*)
6	$4\text{-}ClC_6H_4$	1.0	$Rh_2(OAc)_4$	70	3:1	88 (*R*,*R*)
7	$4\text{-}ClC_6H_4$	0.2	$Cu(acac)_2$	44	3:1	85 (*R*,*R*)

Figure 8. CAA mediated by sulfur ylides using a chiral sulfide.

CONCLUSION

Recent advances in CAA discussed above include two major pathways, addition of a nitrene to an olefin or addition of a carbenoid to an imine. The catalysts reported for these processes are not perfect and in most cases give, at best, only modest yields and ee. No general and efficient procedure has been found for these processes, and the reported ones need to be optimized for every substrate individually. Only a single efficient nitrene source (PhINTs) for the nitrene transfer reaction has been reported. On the contrary, there is not a lack of different diazo compounds for the addition of carbenoids to imines which makes this route synthetically more appealing. The mechanistic rational of both addition processes needs to be clarified. Due to the absence of a clear mechanistic picture, it is at present difficult to rationally approach catalyst design. Clearly, advances in mechanistic understanding will greatly benefit the development of more efficient and selective catalysts.

REFERENCES

(1) (a) Tanner, D. *Angew. Chem. Int. Ed. Engl.* **1994**, 33, 599. (b) Kemp, J. E. G. In *Comprehensive Organic Synthesis*; Trost, B. M.; Fleming, I., Ed.; Pergamon, Oxford, 1991, Vol. 7, pp 469-483.

(2) (a) Mahy, J. P.; Bedi, G.; Battioni, P.; Mansuy, D. *J. Chem. Soc. Perkins Trans. II* **1988**, 1517. (b) Mansuy, D.; Mahy, J. P.; Dureault, A.; Bedi, G.; Battioni, P. *J. Chem. Soc., Chem. Commun.* **1984**, 1161. (c) Mahy, J. P.; Bedi, G.; Battioni, P.; Mansuy, D. *Tetrahedron Lett.* **1988**, 29, 1927.

(3) (a) Groves, J.; Takahashi, T. *J. Am. Chem. Soc.* **1983**, 105, 2073. (b) Mahy, J. P.; Battioni, P.; Mansuy, D.; Fischer, J.; Weiss, R.; Mispelter, J.; Morgenstern-Badarau, I.; Gans, P. *J. Am. Chem. Soc.* **1984**, 106, 1699.

(4) Mahy, J. P.; Battioni, P.; Mansuy, D. *J. Am. Chem. Soc.* **1986**, 108, 1079.

(5) O'Conner, K.; Wey, S.; Burrows, C. *Tetrahedron Lett.* **1992**, 33, 1001.

(6) Noda, K.; Hosoya, N.; Irie, R.; Ito, Y.; Katsuki, T. *Synlett* **1993**, 469.

(7) Nishikori, H.; Katsuki, T. *Tetrahedron Lett.* **1996**, 37, 9245.

(8) Kwart, H.; Khan, A. A. *J. Am. Chem. Soc.* **1967**, 89, 1951.

(9) (a) Evans, D.; Faul, M.; Bilodeau, M. *J. Am. Chem. Soc.* **1994**, 116, 2742. (b) Evans, D.; Faul, M.; Bilodeau, M. *J. Org. Chem.* **1991**, 56, 6744.

(10) Knight, J. G.; Muldowney, M. P. *Synlett* **1995**, 949.

(11) (a) Lowenthal, R.; Masamune, S. *Tetrahedron Lett.* **1991**, 32, 7373. (b) Harm, A.; Knight, J.; Stemp, G. *Synlett* **1996**, 677. (c) Harm, A.; Knight, J.; Stemp, G. *Tetrahedron Lett.* **1996**, 37, 6189.

(12) Perez, P.; Brookhart, M.; Templeton, J. L. *Organometallics* **1993**, 12, 261.

(13) Shi, M.; Itoh, N.; Masaki, Y. *J. Chem. Research* **1996**, 353.

(14) (a) Evans, D.; Woerpel, K.; Hinman, M.; Faul, M. *J. Am. Chem. Soc.* **1991**, 113, 726. (b) Evans, D.; Faul, M.; Bilodeau, M.; Anderson, B.; Barnes, D. *J. Am. Chem. Soc.* **1993**, 115, 5328.

(15) Li, Z.; Conser, K.; Jacobsen, E. *J. Am. Chem. Soc.* **1993**, 115, 5326.

(16) Müller, P.; Baud, C.; Jacquier, Y.; Moran, M.; Nageli, I. *J. Phys. Organic Chem.* **1996**, 9, 341.

(17) Müller, P.; Baud, C.; Jacquier, Y. *Tetrahedron* **1996**, 52, 1543.

(18) Zhang, W.; Lee, N. H.; Jacobsen, E. *J. Am. Chem. Soc.* **1994**, 116, 425.

(19) Li, Z.; Quan, R.; Jacobsen, E. *J. Am. Chem. Soc.* **1995**, 117, 5889.

(20) Aires-de-Sousa, J.; Lobo, A.; Prabhakar, S. *Tetrahedron Lett.* **1996**, 37, 3183.

(21) (a) Zhu, Z.; Espenson, J. *J. Org. Chem.* **1995**, 60, 7090. (b) Zhu, Z.; Espenson, J. *J. Am. Chem. Soc.* **1996**, 118, 9901.

(22) Rasmussen, K.; Jorgensen, K. *J. Chem. Soc., Chem. Commun.* **1995**, 1401.

(23) Hansen, K.; Finney, N.; Jacobsen, E. *Angew. Chem. Int. Ed. Engl.* **1995**, 34, 676.

(24) Moran, M.; Bernardinelli, G.; Müller, P. *Helv. Chim. Acta* **1995**, 78, 2048.

(25) Aggarwal, V.; Thompson, A.; Jones, R.; Standen M. *J. Org. Chem.* **1996**, 61, 8368.

CHEMICALLY USEFUL ENZYMATIC REACTIONS IN ORGANIC SOLVENTS

Reported by David J. Schenk Thursday April 24, 1997

INTRODUCTION

The recognition that enzymes are stable in organic solvents has led to a wide range of synthetically useful transformations. Using organic solvents for enzymatic reactions can provide advantages over performing reactions in water.[1,2] The increased solubility of organic substrates is often observed. Under these conditions the potential for substrate or product inhibition can be decreased.[1,2] In addition, the substrate can be used as the bulk organic phase.[2] In other cases the removal of the aqueous phase can modify the equilibrium position of the reaction e.g. favoring condensation reactions over hydrolysis.[1,2]

When there is no aqueous phase then a change in solvent can change many features of an enzymatic reaction such as regioselectivity, substrate specificity, enantioselectivity, enzyme stability, and rate.[1b,2] Other reasons for the use of enzymes in organic solvents are ease of product purification, synthetically practical rates, and likely environmental advantages over metallic chemical reagents.[1b,2] This review will cover only heterogeneous, cell-free enzymatic systems in organic solvents with no bulk aqueous phase.

PROPERTIES OF ENZYMES IN ORGANIC MEDIA

Enzyme Stability / Activity

The environment of an enzyme in an organic solvent is considerably different from that in aqueous solution. Many of the forces that hold an enzyme together arise from interactions with water,[1a] but enzymes have been shown to have approximately the same structure in a solvent (non-protein dissolving) as they do after lyophilization.[3] With the change from aqueous to organic solvent, the salt bridges and ionic interactions responsible for the enzymes structure are stronger than in aqueous solution.[2a] These added forces impart the enzyme with increased stability.[1b,2] Also, since most microorganisms can not survive in organic solvents, and thus can not form proteases, organic solvents provide further stability to enzymes.[1b] In some cases the enzymes presence in solvents can also lead to increased thermal stability.[1b] For example porcine pancreatic lipase was stable in heptanol for 10 min at 100 °C while in water at this temperature it was denatured immediately.[4]

Copyright © 1997 by David J. Schenk

Enzymes are generally less active in organic solvents, not because of solvent denaturing, but because of conformational changes during lyophilization,[3] the unfavorable partitioning of the substrate from organic solvent to enzyme surface and active site, and loss of enzyme flexibility due to increases in salt bridge strength.[5] Generally, enzyme structures in anhydrous solvents are more similar to those in water[3] than in water/organic mixtures.[6]

Water Activity

In anhydrous solvents, the activity of an enzyme can be drastically affected by the nature of the solvent.[1,2] Enzymes require some minimal level of water for activity, and in many cases only a monolayer of water on the surface of the enzyme is necessary.[1b,2a] In polar solvents this layer is removed thus lowering the activity or inactivating the enzyme.[1b,2] Non-polar solvents cause minimal removal of this layer, since they have low water solubility, thus retaining the enzymes activity. Often the use of a buffer-saturated organic solvent, to provide consistent water concentration from experiment to experiment is useful. Increases in the reproducibility of enzymatic activity are then observed, since the activity of an enzyme is related to the amount of water near it.[2]

Enzyme Forms

Many different enzyme types can be considered for use in organic solvents: powdered enzymes, soluble derivatives, and covalently cross-linked enzymes crystals.[2] Often different forms of an enzyme, will have different stability, and in some cases different selectivities.[2]

Effect of Processing "Memory Effect" / Substrate Effect

The activity of an enzyme in an organic solvent is affected by a number of parameters. One of the most surprising is the "memory" of an enzyme for the pH of the last aqueous solution that it has been in contact.[2] If an enzyme is lyophilized from solutions of different pHs then put in an organic solvent, there will be a relationship between this pH and enzymatic activity. The cause of this memory effect is that at different pHs the active site, and the whole protein, can have different structures, and once removed from the aqueous phase they are locked into this structure.[2]

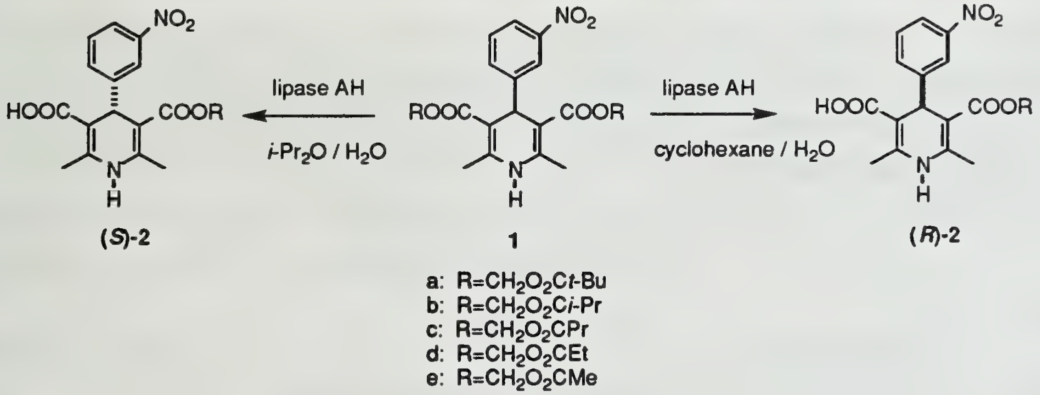
The selectivity of enzymes can be modified by previous exposure to a substrate.[2] If an enzyme is lyophilized in the presence of a substrate mimic, then placed in an organic solvent the structure of the active site is modified to fit this binding substrate.[2] Once the substrate mimic is washed away, the enzyme will now possess a modified active site for this shape of substrate, and it can not revert to the original structure, unless it is subsequently dissolved in an aqueous solvent.

Solvent Effects on Selectivity

Enantioselectivity. Solvents can have drastic effects on the enantioselectivity of enzymes[2,8], and in some cases the inversion of enantioselectivity is observed.[9] Models relating selectivity to solvent parameters have been proposed.[8] The reversal of enantioselectivity of lipase AH (*Pseudomonas sp.*) catalyzed hydrolysis of diesters **1** was observed (Table 1).[10] For the mutant FVL lipase PS hydrolysis of other 1,4-dihydropyridines, a change of solvent from isopropyl ether to cyclohexane, both saturated with water, also resulted in the inversion of enantioselectivity from *S* with >99 ee % to *R* with 45 ee %.[11a]

Colvalently cross-linked enzyme crystals of γ-chymotrypsin also show an inversion in selectivity for the transesterification of methyl-3-hydroxy-2-phenylpropanoate at low conversion.[11b] The enantioselectivity of *Aspergillus oryzae* protease showed an approximately linear correlation with log P of the solvent.[8b] With this relationship of log P to selectivity, a model for the active site was proposed which on further study with modified substrates was verified.[8b]

Table 1. Effect of Solvent on the Enantioselectivity of Lipase AH[10]



	i-Pr2O (H2O saturated)				cyclohexane (H2O saturated)			
substrate	time, h	yield, %	ee, %	config. 2	time, h	yield, %	ee, %	config. 2
1a	48	87	>99.0	*S*	48	88	88.8	*R*
1b	24	83	89.0	*S*	48	71	91.0	*R*
1c	24	71	88.9	*S*	48	62	91.1	*R*
1d	1	83	68.1	*S*	17	57	91.4	*R*
1e	5	77	42.3	*S*	17	32	88.2	*R*

Conditions: 25 mg substrate , 25 mg lyophilized lipase, and 1 mL solvent saturated with water at 20°C.

Selectivity inversions are believed to be uncommon, though many examples of effects of solvent on enantioselectivity are known.[9b] In the resolution of α–methylbenzylamine a dependence of relative reaction rate on solvent was observed.[9b] An effect of the pressure of supercritical fluids on enzymatic enantioselectivity of subtilisin Carlsberg catalyzed esterification, has been observed for *N*-acetyl-phenylalanine ethyl ester owing to the change in solvent polarity with pressure.[12]

Regioselectivity. *Pseudomonas cepacia*, and *Pseudomonas* lipoprotein lipases showed reversals in regioselectivity between toluene and acetonitrile for substituted 1,4-butyl diesters (Scheme 1).[13] A model proposing location and size of binding sites was tested by using substituted substrates. A near linear relationship between selectivity and log P of the solvent for the transesterification of dibutyrylated octylhydroquinones with *Pseudomonas cepacia* lipase was found.[14] For *Chromobacterium viscosum*, *Candida cylindracea*, *Aspergillus niger* and for nonenzymatic transesterifcation the least hindered alcohol was provided.

Prochiral / Meso Selectivity. For substituted 2-benzyl 1,3-propanediols, theoretical correlations of prochiral selectivity (k_{cat}/K_m) vs. thermodynamic activity coefficients of the desolvated substrate were developed.[8a] For glutaraldehyde cross-linked enzyme crystals of γ-chymotrypsin, or subtilisin Carlsberg-catalyzed acylations, the above dependence on solvent were followed well, while for lyophilized or precipitated enzyme they are less well correlated. Also a solvent-dependent reversal of prochiral selectivity was observed for the acylation of a 2-benzyl 1,3-propanediol (Table 3).

Scheme 1

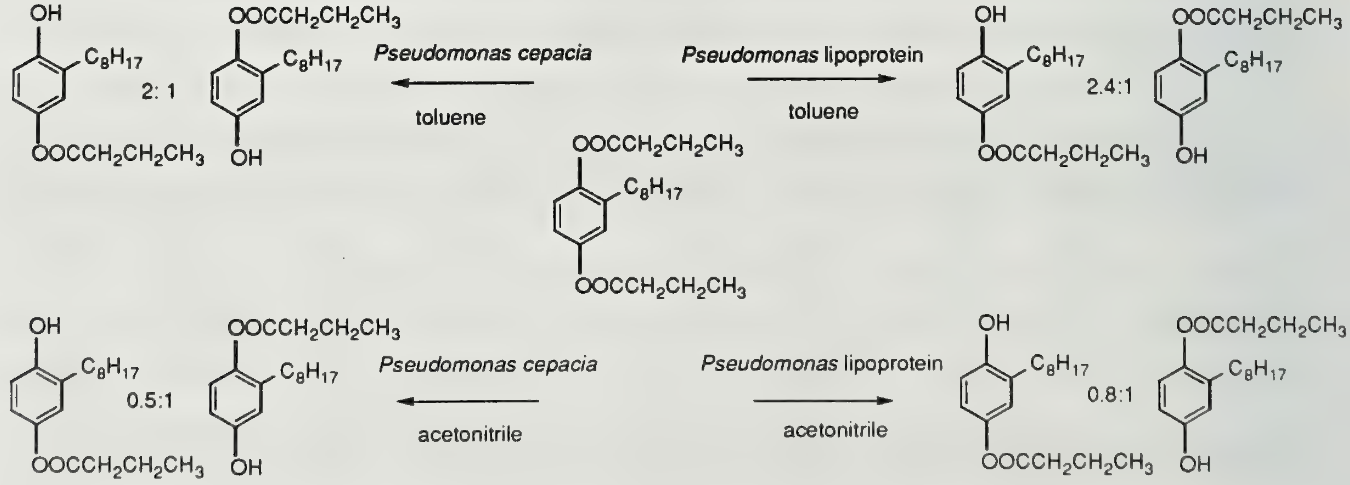
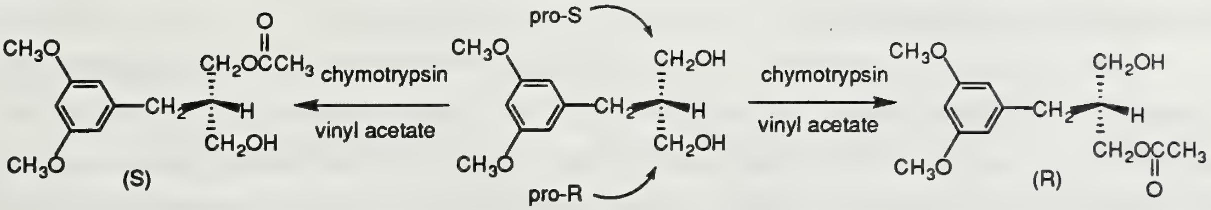


Table 3. Prochiral Selectivity $(k_{cat}/K_m)_{pro\text{-}S}$ / $(k_{cat}/K_m)_{pro\text{-}R}$ Dependence of γ-Chymotrypsin in Organic Solvents[8a]



	$(k_{cat}/K_m)_{pro\text{-}S}/(k_{cat}/K_m)_{pro\text{-}R}$		
solvent	cross-linked crystals[a]	after lyophilization[b]	acetone precipitated[b]
diisopropyl ether	0.10	0.98	0.63
cyclohexane	0.28	0.91	0.67
p-xylene	1.0	1.3	NA
acetonitrile	1.9	2.9	1.0
methyl acetate	2.2	1.2	1.5

Conditions: 100mM vinyl acetate, 1mL solvent, 10 mM diol, 0.2% (v/v) water, 45°C. a) 5 mg enzyme. b) 15 mg enzyme.

Substrate Specificity

Inversions in substrate selectivity of acylated amino ester hydrolysis in water compared to transesterification in octane have been observed for protected amino esters.[17a] Theoretical models based on enzyme kinetics have been developed to explain this phenomenon.[9a] A significant solvent effect on the nucleophilicity of alcohols was observed for subtilisin-catalyzed transesterification of *N*-acetyl-L-phenylalanine ethyl ester.[17b]

EXAMPLES OF USE

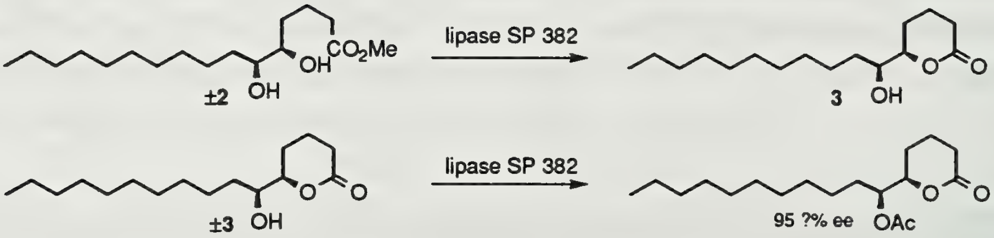
Bond Formation / Functional Group Transformations

A wide range of synthetically useful reactions has been performed with enzymes in near anhydrous systems.[2] Some of the most significant reactions are those which proceed in the reverse direction (condensation) owing to the water affinity of anhydrous solvents.[2] Examples include the synthesis of peptides,[18] sugars, and sugar derivatives,[19] and polymers.[19a,20]

The synthesis of the octapeptide, Lys-Gly-Asp-Glu-Glu-Ser-Leu-Ala, beginning with the enzymatic coupling of *N*-Cbz-protected ethyl esters of lysine, and glutamic acid proceeded with overall yield of 39%.[18b] The avoidance of C-deprotection and activation steps, normally required for peptide synthesis, and the absence of competing hydrolysis and racemization[21] which often occurs in enzymatic reactions in aqueous solution, provides potential advantages for this technique. Additionally, non-natural D-amino acids can be incorporated to produce dipeptides by enzymatic reactions in *t*-amyl alcohol.[22a] Normally little activity is seen for enzymes with D-amino acids.[22b]

Other synthetic reactions include oxidation,[14] functional group transformations,[23] acylation,[24] and lactonization.[25] A racemic dihydroxy ester was selectively lactonized with pancreatin or lipase SP 382 in *t*-butyl methyl ether to give after purification enantiomerically pure lactone.[25a] Also, selective acylation of the racemic hydroxy lactone proceeded with 95 % ee.

Scheme 2

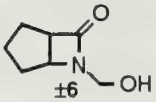


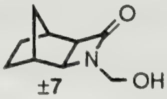
The synthesis of (*R*,*R*)-(-)-pyrenophorin (**4**) (Scheme 3) began with a yeast-catalyzed reduction[25b] (*Pichia farinosa*) followed by lipase resolution[26] in toluene, afforded alcohol **5** in 97% ee. *Candida antarctica* lipase-mediated lactonization in isooctane[25b] produced the dimer in 44% yield with 32% of trimeric lactone which was recycled.

(*R*) or (*S*)-Oxynitrilase catalyzed hydrocyanation of arenecarbaldehydes using almond meal, or dechlorophylled shoots from *Sorghum bicolor* produced either (*R*) or (*S*) cyanohydrins, respectively. The yield and ee for some substituted arenecarbaldehydes was as high as those with the purified enzymes.[23a] Also purified (*R*) or (*S*)-Oxynitrilase afforded cyanohydrins from a variety of arenecarbaldehydes in good yields and ee.

Resolutions

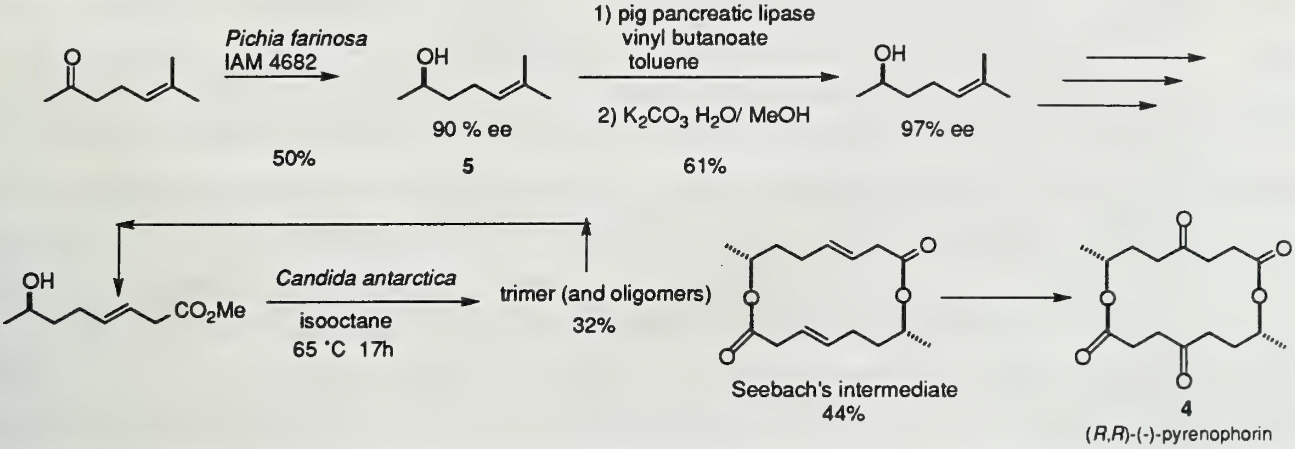
Resolutions by acetylation of bicyclic and tricyclic β-lactams (**6**,**7**), as precursors to β-amino acids, were accomplished in acetone with Celite adsorbed lipase AK giving high ee's of both products and resolved starting materials.[27] In a related work esterifications catalyzed by lipases PS and SP 526 produced the related β-amino acid esters from the racemates.[28]


±6


±7

Alcoholysis of *N*-substituted aziridine carboxylates with pig pancreatic or *Candida cylindracea* lipases in hexane afforded good yields (80-98%) for the cleavage of the N-R bond.[29] In lipase PS-catalyzed acylation or deacylation of a variety of 1-phenylethanols, 1-phenylethanoates, and 1-phenyl substituted cyanohydrins proceeded with good yields and enantiomeric excesses.[23a]

Scheme 3



Regioselective acylation of 1,3,5-O-methylylidyne-*myo*-inositol with vinyl acetate in THF, and *Pseudomonas* lipoprotein lipase, followed by benzyl chloride protection and hydrolysis of the acetate gave the diprotected *myo*-inositol in 80 % yield. *Pseudomonas* lipoprotein lipase enantioselective esterification of the diprotected *myo*-inositol with vinyl butyrate in acetone gave selectivly the triprotected product in >95% ee.[19c,30] Selective acylation, benzylation, deprotection, and phosphorylation and deprotection produced D-*myo*-inositol-1,2,6, triphosphate.

CONCLUSION

The ability of enzymes to function in organic solvents provides the ability to fine-tune reactions toward a certain product without resorting to developing mutant enzymes. Once an enzyme has been shown to produce a product in water, then the optimal pH for lyophilization can be found and the effect of solvent determined. In some cases the water affinity of an organic solvent can shift the equilibrium giving a needed product, while in water side reactions may compete. A number of reaction types besides resolution can be performed giving a wide scope to the use of enzymatic reactions in organic solvents.

References

(1) (a) Chen, C.-S.; Sih, C. J. *Angew. Chem. Int. Ed. Engl.* **1989**, *28* 695. (b) Klibanov, A. M. *ChemTech* **1986**, *16*, 354.

(2) *Enzymatic Reaction in Organic Media*; Koskinen, A. M. P.; Klibanov, A. M., Ed.; Blackie Acad. and Professional: New York, NY, 1996.

(3) Desai, U. R.; Klibanov, A. M. *J. Am. Chem. Soc.* **1995**, *117*, 3940.

(4) Zaks, A.; Klibanov, A. M. *Science* **1984,** *224* , 1249.

(5) Schmitke, J. L.; Wescott, C. R.; A. M. Klibanov; *J. Am. Chem. Soc.* **1996**, *118*, 3360.

(6) Griebenow, K.; Klibanov, A. M. *J. Am. Chem. Soc.* **1996**, *118*, 11695.

(7) Xu, K.; Kilbanov, A. M. *J. Am. Chem. Soc.* **1996**, *118*, 9816.

(8) (a) Ke, T.; Wescott, C. R.; Klibanov, A. M. *J. Am. Chem. Soc.* **1996**, *118*, 3366. (b) Tawaki, S.; Klibanov, A. M.; M. *J. Am. Chem. Soc.* **1992**, *114*, 1882.

(9) (a) Wescott, C. R.; Klibanov, A. M; *J. Am. Chem. Soc.* **1993**, *115*, 10362. (b) Kitaguchi, H.; Fitzpatrick, P. A.; Huber, J. E.; Klibanov, A. M.; *J. Am. Chem. Soc.* **1989**, *111*, 3094.

(10) Hirose, Y.; Kariya, K.; Sasaki, I.; Kurono, Y.; Ebiike, H.; Achiwa, K. *Tetrahedron Lett.* **1992**, *33*, 7157.

(11) (a) Hirose, Y.; Kariya, K.; Nakanishi, Y.; Kurono, Y.; Achiwa, K. *Tetrahedron Lett.* **1995**, *36*, 1063. (b) Wescott, C. R.; Noritomi, H.; Klibanov, A. M. *J. Am. Chem. Soc.* **1996,** *118*, 10365.

(12) Kamaat, S. V.; Beckman, E. J.; Russel, A. J.; *J. Am. Chem. Soc.* **1993**, *115*, 8845.

(13) Rubio, E.; Fernandez-Mayorales, A.; Klibanov, A. M.; *J. Am. Chem. Soc.* **1991**, *113*, 695.

(14) Kazandjian, R. Z.; Klibanov, A. M. *J. Am. Chem. Soc.* **1985**, *107*, 5448

(15) Harris, K. J.; Gu, Q.-M.; Shih, Y.-E.; Gardaukas, G.; Sih, C. J. *Tetrahedron Lett.* **1991**, *32*, 3941.

(16) Guo, Z.-W.; Wu, S.-H.; Chen, C.-S.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1990**, *112*, 4942.

(17) (a) Zaks, A.; Klibanov, A. M. *J. Am. Chem. Soc.* **1986**, *108*, 2767. (b) Chaudhary, A. K.; Kamat, S. V.; Beckman, E. J.; Nurok, D.; Kleyle, R. M.; Hajdu, P.; Russell, A. J. *J. Am. Chem. Soc.* **1996**, *118*, 12891.

(18) Gill, I.; López-Fandino, R.; Vulfson, E. *J. Am. Chem. Soc.* **1995**, *117*, 6175.

(19) (a) Martin, B. D.; Ampofo, S. A.; Linhardt, R. J.; Dordick, J. S. *Macromolecules* **1992**, *25*, 7082. (b) Wulff, G.; Schmid, J.; Venhoff, T. *Macromol. Chem. Phys.* **1996**, *197*, 259.

(20) (a) Svirkin, Y. Y.; Xu, J.; Gross, R. A.; Kaplan, D. L.; Swift, G. *Macromolecules* **1996**, *29*, 4591. (b) Xu, Jin, Gross, R. A.; Kaplan, D. L.; Swift, G. *Macromolecules* **1996**, *29*, 4582.

(21) Hwang, B. K.; Gu, Q.-M.; Sih, C. J. *J. Am. Chem. Soc.* **1993**, *115*, 7912.

(22) (a) Margolin, A. L.; Tai, Dar-Fu; Klibanov, A. M. *J. Am. Chem. Soc.* **1987**, *109*, 7885. (b) See exceptions Ref. 24a.

(23) (a) Kanerva, L. T. *Acta Chem. Scan.* **1996**, *50*, 234. (b) Yang, F.; Russell, A. J.; *Biotechnol. Prog.* **1993**, *9*, 234;

(24) Review Faber, K.; Riva, S. *Synthesis* **1992**, 895.

(25) (a) Henkel, B.; Kunath, A.; Schick, H. *Liebigs Ann.* **1995**, 921. (b) Sugai, T.; Katoh, O.; Ohta, H. *Tetrahedron*, **1995**, *51*, 11987.

(26) Sugai, T.; Ohta, H. *Agric. Biol. Chem.* **1990**, *54*, 1577.

(27) Csomós, P.; Kanerva, L. T.; Bernáth, G.; Fülöp, F. *Tetrahedron Asym.* **1996**, *7*, 1789.

(28) Kanerva, L. T.; Csomós, P.; Sundholm, O.; Bernáth, G.; Fülöp, F. *Tetrahedron Asym.* **1996**, *7*, 1705.

(29) Martres, M.; Gil, G.; Méou, A. *Tetrahedron Lett.* **1994**, *35*, 8787.

(30) Andersch, P.; Schneider, M. P. *Tetrahedron Asym.* **1993**, *4*, 2135.

ALLYLINDIUM REAGENTS IN ORGANIC SYNTHESIS

Reported by Corey Stanchina May 1, 1997

INTRODUCTION

Allylmetallations have found extensive use over the years as one of the primary methods of C-C bond formation.[1] Various metals such as Al, Zn, Sn, B, Si, Mg, and Li have been employed for this purpose. However, several problems arise from the use of these metals. Enolization or reduction of aldehydes compete with allylation, often resulting in only moderate yields. Complex mixtures of Wurtz-type side products[2] are also observed under these reaction conditions. Strong Lewis acids such as $TiCl_4$ are sometimes necessary to promote the allylations.[2] In addition, the sensitivity of these reagents to water restricts their use to rigorously anhydrous conditions and heightens the complexity of the protocol. With these allylmetals, substrate diversity is usually limited to aldehydes, ketones, and some imines, alkenes, and alkynes.[2]

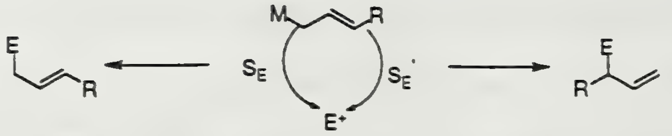
Recently, indium has been employed in allylations of various classes of substrates including alkynols,[3] imides,[4] anhydrides,[5] and 1,3-dicarbonyls.[6] Not only do allylindium reagents offer the synthetic utility that is common among the metals listed above, but they also extend these benefits to include applications in aqueous systems. The reaction protocols for indium-mediated allylations are very simple, often providing the corresponding products with high regio- and diastereoselectivities in both anhydrous and aqueous conditions. Indium-mediated allylations have also recently been applied to the synthesis of carbohydrates.[7-11] Since these allylations are done using water as the solvent, hydroxyl protecting groups are unnecessary. Unlike allylzinc and allyltin reagents in carbohydrate syntheses, indium does not require acid catalysis,[12] heat,[13] or sonication[14] to promote the reaction.

The physical properties of indium differ greatly from the metals listed above. It has been shown that indium does not readily form oxides in air and is unaffected by boiling water or alkali.[15] It has the lowest first ionization potential among Al, Mg Zn, and Sn, but the highest second ionization potential.[7] However, the limiting factors of its use are the cost (ca. \$3/g) and its moderate level of toxicity.[16] The issue of cost becomes less of a factor since indium can be regenerated electrochemically.[17] This report summarizes and describes the synthetic utility of allylindium reagents both in aqueous and organic solvents.

MECHANISTIC CONSIDERATIONS

Allylic additions to carbonyls and various other substrates is thought to proceed via an S_E' or an S_E mechanism in organic solvent (eq 1).[2] However, in aqueous media a single electron transfer (SET)

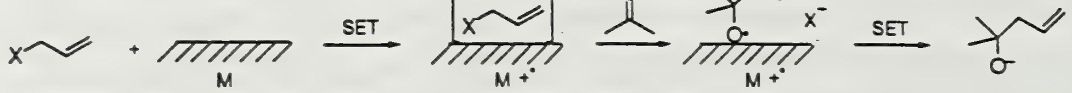
Copyright© 1997 by Corey Stanchina



(1)

mechanism has been proposed (Scheme 1). The effectiveness of indium in these aqueous reactions may be due in part to its relatively low first ionization potential and high second ionization potential.[18]

Scheme 1

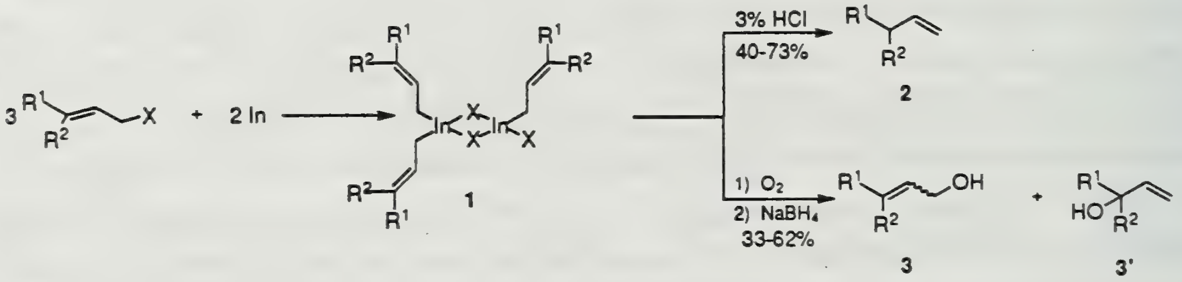


ALLYLATIONS IN ORGANIC MEDIA

Generation of Allylindium Sesquihalide

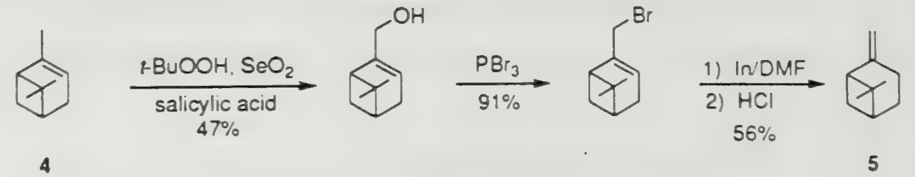
Indium-mediated allylation of various carbonyl substrates, which proceeds through a common intermediate indium species has been investigated. Spectroscopic evidence (^{1}H NMR and IR) of the nature of this intermediate suggests an allylindium sesquihalide (**1**) formed by reaction of indium with the allyl halide (preferably an allylic iodide or bromide) in a 2:3 ratio[1,3] (Scheme 2). The ^{1}H NMR spectrum (DMF-d_7, -30°C) of this intermediate revealed two sets of allylic signals in a ratio of 2:1, consistent with **1**.

Scheme 2



Protonolysis of **1** in 3% aqueous HCl gave the γ-protonated alkenes exclusively with a transposition of the allylic bond.[4] All the allyl halides investigated thus far[4] gave high selectivity (>99%) in formation of the terminal olefin (Scheme 2). This method was utilized in the conversion of α-pinene, **4**, to β-pinene, **5**, in 24% overall yield (Scheme 3).[4]

Scheme 3



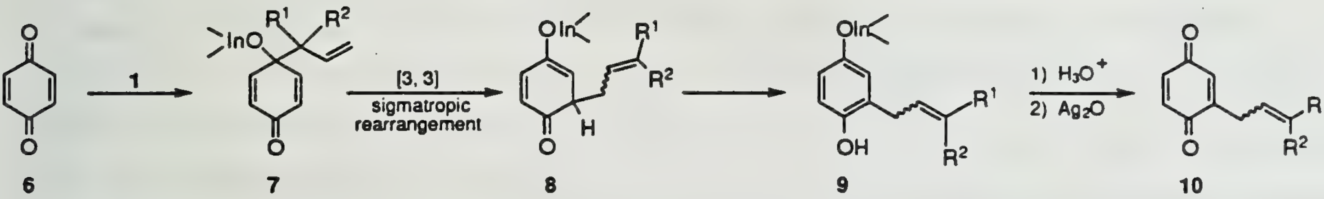
4 5

Generally, oxygenation of allylmetal species provides complex mixtures of products.[19] However, oxygenation of the air-sensitive sesquihalide **1** followed by reduction with $NaBH_4$ afforded isomeric allylic alcohols in reasonable combined yields (Scheme 2).[4]

Reaction with Carbonyls

Allylindium sesquihalide **1** has been shown to undergo 1,2-addition to a wide range of aldehydes and ketones, both aromatic and nonaromatic, in high yields and under very mild conditions.[20] In addition, a variety of quinones have also been allylated using **1**.[21] Allylations of unsubstituted 1,4-benzoquinones with allylindium, prenylindium, geranylindium, and nerylindium reagents gave good to excellent yields of the allylated quinone after oxidation with Ag_2O. Complete scrambling of the geometry around the double bond in the neryl- and geranylindium-induced allylations suggests γ-addition of the allylic indium sesquihalide, followed by a [3, 3]-sigmatropic rearrangement (Scheme 4).

Scheme 4

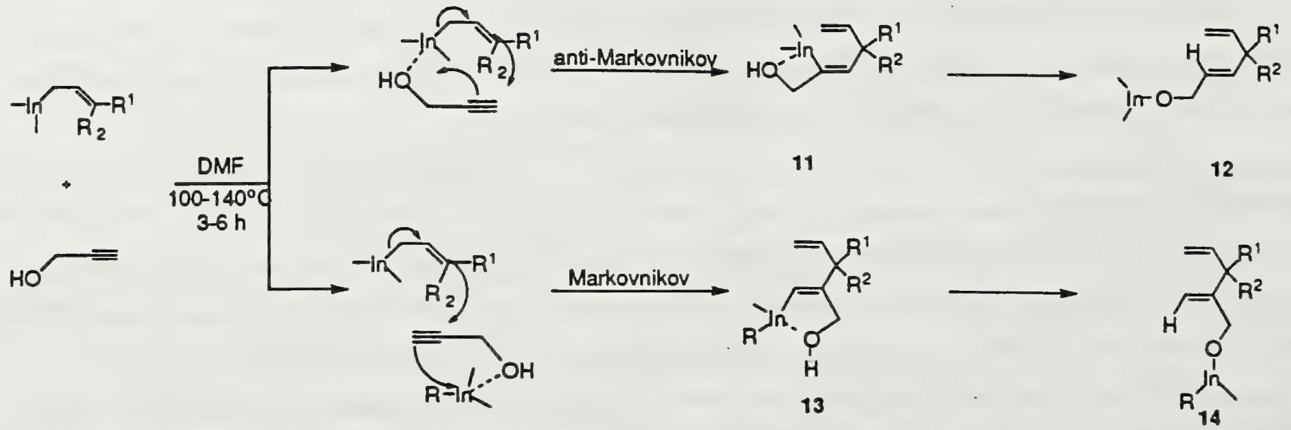


Substituted *p*-benzoquinones gave the corresponding sterically less hindered quinones. In all cases investigated, prenylation and geranylation of the of the *p*-quinones favored the monoallylated products over the diallylated products. Varying the amounts of quinone had a substantial effect on the mono- versus diallylated product ratio. Substituted and unsubstituted haloquinones are readily prenylated to afford modest to excellent yields of the allylated products. Cinnamylation and prenylation of hydroxyquinones also afforded the corresponding hydroxyquinones in good yields. The electron donating nature of the oxygenated substituent deactivates the C(4) carbonyl carbons forcing the indium reagent to orient itself around the more sterically hindered C(1) carbonyl.

Reaction with Alkynes

Terminal alkynes bearing a neighboring hydroxyl moiety undergo facile allylation with **1** between 100-140°C, giving 1,4-dienes.[22] The determination of whether the *anti*-Markovnikov or the Markovnikov product is formed is governed largely by the structures of both the allylindium reagent and the alkyne (Scheme 5). Mechanistic studies suggest that the *anti*-Markovnikov product is formed by a process analogous to allylboration;[23] the hydroxyl functionality coordinates to the indium atom forming a 4- or 5-membered ring intermediate **11**, thus facilitating allylation. Alternatively, Markovnikov allylation may take place *via* an intermolecular process also shown in Scheme 5. In a manner similar to allylmagnesiation of alkynols[24] an external indium atom may provide a coordination site to facilitate allylation. An intramolecular process in formation of the *anti*-Markovnikov product would be sterically

Scheme 5

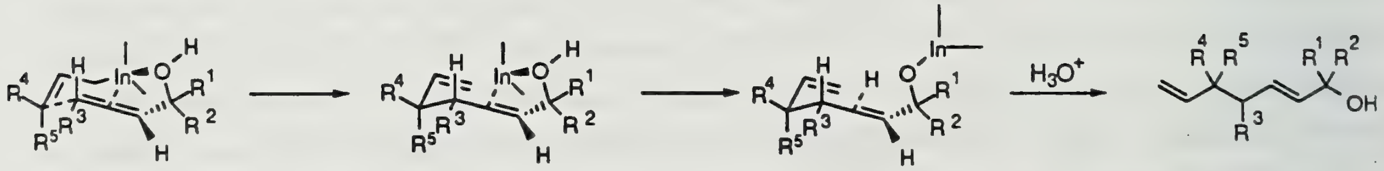


disfavored. This method has been employed in concise syntheses of yomogi alcohol, achillenol and isomyrcenol.[22]

Reaction with Allenols

A variety of allenols have been shown to undergo allylation with **1** in a one-pot synthesis of 1,5-dienes.[25] Substituted allenols gave modest diastereoselectivities of the corresponding dienes in reasonable yields. A neighboring hydroxyl group has been shown to be essential for the reaction to proceed. Scheme 6 depicts the rationale behind the selectivity of the reaction. Chelation of the indium atom with C-(3) carbon and the oxygen atom of the allenol leads to a bicyclic transition state. The mechanism is exclusively intramolecular compared to other allylation methods of allenes which proceed both inter- and intramolecularly.[26]

Scheme 6

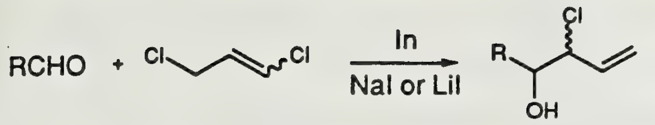


Other Allylindinations

Recently cyclic anhydrides,[5] imides,[4] and cyclic imides,[4] have been shown to undergo allylations with **1** to give both mono- and diallylated products in good to excellent yields depending on the degree of substitution of the allylindium reagent. However, diastereomeric ratios as well as the stereochemical ratios were relatively low.

Generation of chlorohydrins from the condensation of 1,3-dichloropropene with aldehydes proceeds in good to excellent yields and in high syn/anti ratios[27] (Scheme 7). It has been shown that these allylations do not occur in the absence of LiI or NaI, which is needed to aid in the formation of the indium sesquihalide **1**. Temperature and solvent effect studies revealed that the highest syn/anti selectivities occurred at temperatures between -60 °C and -30 °C in polar solvents (DMF and THF).

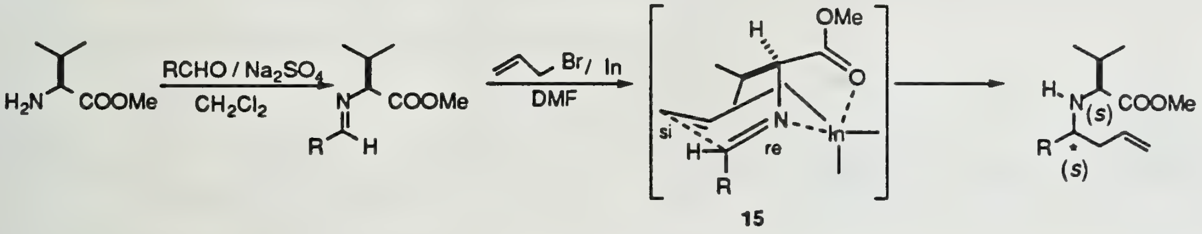
Scheme 7



solvent	temp., °C	yield, %	syn / anti
THF	0	88	79 / 21
DMF	-60 to -30	83	92 / 8

More recently, the asymmetric synthesis of homoallylic amines has been achieved with high stereoselectivity in the presence of **1**.[28] The methyl ester of L-valine was reacted with an aldehyde to afford an imine. Allylindination of the imine with allylbromide provided the enantiomerically enriched amino acid with an (*S/S*, *S/R*) diastereomeric ratio of >9/1 (Scheme 8). The reaction proceeds through a rigid N, O-bidendate nature of the chelated imine **15** in the transition state. Consequently, allylation directed to the *re*-face is less preferred owing to the bulky isopropyl group, thus giving predominately *si*-face addition.

Scheme 8



INDIUM-MEDIATED ALLYLATIONS IN AQUEOUS MEDIA

Reaction with Aldehydes

Reactions of γ-substituted allyl bromides were carried out with various aldehydes in water.[30] Regio- and diastereoselectivities were shown to be governed by the nature of the substituents on both the aldehyde and the allylbromide. Cyclic transition state structures **25a** and **25b** represent the preferred pathway where the γ-substituents on the allyl reagent are bulky, giving rise to the α-adducts. Cyclic transition state structures **26a** and **26b** are favored when the selectivity is governed by the size of the aldehyde substituent (Figure 3).

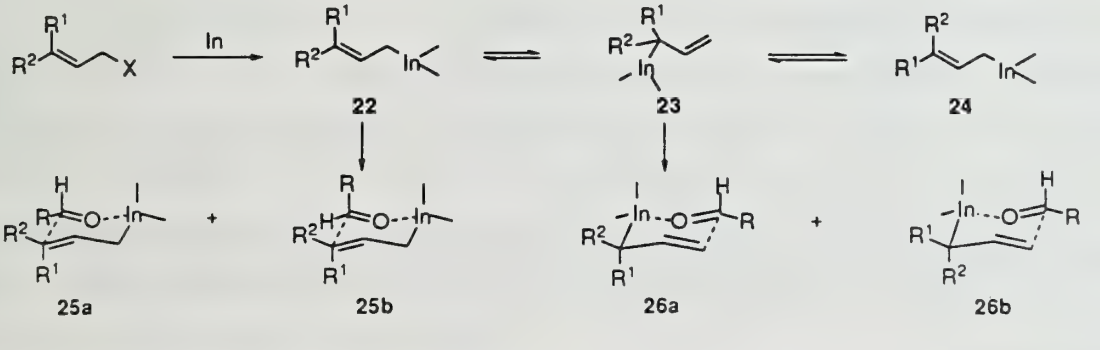
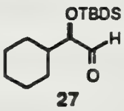
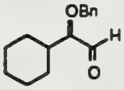


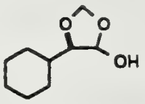
Figure 3. Transition state structures for variations in γ-substituent and aldehyde substituents

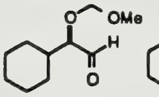
Addition to carbonyls was extended to the reaction of α- and β-oxygenated aldehydes in aqueous and nonaqueous media with allyl bromide and indium metal (Table 1). Allylations performed in H_2O or THF/H_2O mixtures proceed at a more appreciable rate than in THF alone. Diastereoselectivities providing the syn products generally were highest with the α-hydroxy aldehydes and lowest with the aldehydes in which the α-substituent contained a poor chelating group, such as an epoxide or a bulky silyloxy group (Table 1). β-Oxygenated aldehydes behaved in an analogous fashion.

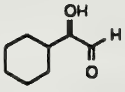
Table 1. Allylations of various aldehydes in H_2O and a 1/1 THF/H_2O mixture

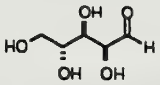

27

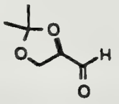

28

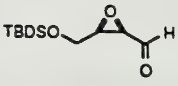

29


30


31


32


33


34

Aldehyde	product ratio (THF-H_2O)		Yield, % (THF-H_2O)	product ratio (H_2O)		Yield, % (H_2O)
	syn	anti		syn	anti	
27	1	4.2	87	1	3.9	90
28	1	2.2	93	1	1.2	92
29	2.3	1	93	2.3	1	95
30	1.7	1	86	2.1	1	85
31	NA	NA	NA	9.8	1	85-90
32	8.2	1	87	10.2	1	90
33	1	3.9	80	1	3.2	83
34	1	2	82	1	2	78

Transition state models **35** and **36** (Figure 4) represent limiting proposals that rationalize the selectivities observed. In these models allyl group transfer to the carbonyl carbon from the less hindered π-surface opposite to the R group occurs (Cram control).

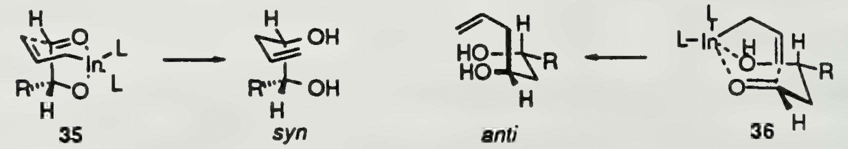
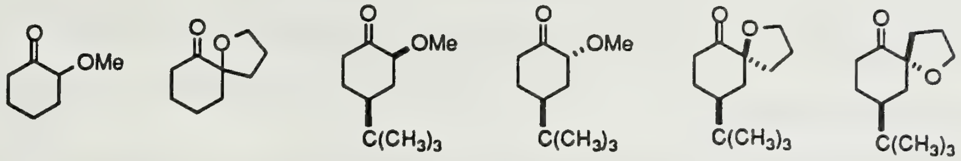


Figure 4. Rationalization of the diastereoselectivities for α- and β-oxygenated aldehydes

In all allylations investigated, the pH of the reaction medium dropped significantly as the reactions proceeded. In some cases, low pH significantly increased the rates of allylation, especially with **29**, where the hemiacetal is hydrolyzed first, then allylated. However, other cases (**27** and **28**) no changes were observed. Generally, a pH dependence on syn/anti ratios was ruled out as a contributing factor in diastereoselectivities. On the other hand, salt effects substantially increased the syn/anti ratios as well as rate of the reaction, presumably due to the increase in ionic strength of the medium.

Reaction with Ketones

Several cyclohexanone systems were studied to determine the extent of substrate chelation with allylindium reagents in aqueous and nonaqueous solvents (Figure 1).[29] Compounds **16** and **17** display the highest syn/anti product ratio (14.1/1) in a 1/1 THF/H_2O solvent mixture and lowest in THF alone (9/1). When locked in a single chair conformation, excellent diastereoselectivity in favor of the syn adduct resulted, as in the case with **18** (>32/1). Ketones **19** and **21** also showed reasonable diastereoselectivities in favor of the syn product presumably due to the chelation-controlled addition of the allyl group *via* twist-boat conformations (Figure 2).



16 **17** **18** **19** **20** **21**

Figure 1. Ketones used in chelation control studies.

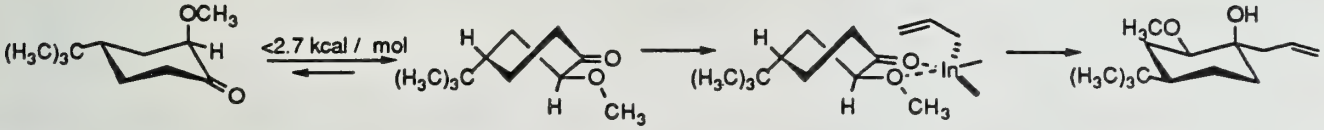
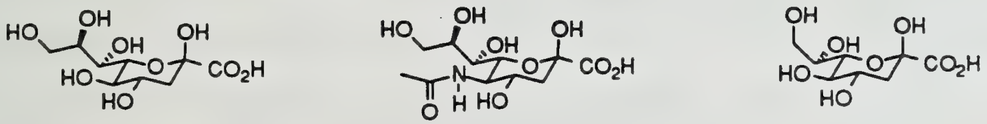


Figure 2. Proposed mechanism of the allylindination of **19**.

Applications

The indium allylation method was utilized in the synthesis of (+)-3-deoxy-D-*glycero*-D-*galacto*-nonulosonic Acid (KDN),[31] **37** (Figure 5), and *N*-acetyl-D-mannosamine (Neu5NAc) **38**[32] in high yields and purity. The carbohydrate 3-*deoxy*-D-*manno*-2-ochilosonic acid (KDO) **39**[33] as well as analogs of (dehydro)-neuraminic acids[30] have been efficiently synthesized with selectivities comparable to other methods.[10] The reaction could be run on a 0.1 mg to ≥ 5 g scale without noticeable changes in yields.



37 **38** **39**

Figure 5. Structures of KDN, Neu5NAc, and KDO

Other applications using allylindium reagents include generation of allylindium reagents in situ in water,[8,34-36] useful formation of allyl gem-dianion synthons,[37] preparation of β-trifluoromethylated homoallylic alcohols,[38] and allenol syntheses.[39]

CONCLUSION

Nucleophilic additions mediated by indium metal have recently become an important addition in the tool box of a synthetic organic chemist. The vast range of applications with indium and its ability to react in both organic and aqueous solvents have expanded the possibilities for its use. With its rapidly expanding utilities, indium may eventually take presedence over the present-day commonly used metals for allylations in organic chemistry.

REFERENCES

(1) Gynane, M. J. S.; Worrall, I. J. *J. Organomet. Chem.* **1974**, *81*, 329-334.
(2) Yamamoto, Y.; Asao, N. *Chem. Rev.* **1993**, *93*, 2207-2293.
(3) Butsugan, Y.; Shimizu, K.; Imai, A.; Araki, S. *Tetrahedron Lett.* **1992**, *33*, 2581-2582.
(4) Butsugan, Y.; Jin, S.J.; Perminder, T. S.; Araki, S. *J. Org. Chem.* **1991**, *56*, 2538-2542.
(5) Butsugan, Y.; Ito, H.; Katsumura, N.; Araki, S. *Tetrahedron Lett.* **1989**, *30*, 1581-1582.
(6) Li, C. J.; Lu, Y.Q. *Tetrahedron Lett.* **1995**, *36*, 2721-2724.
(7) Chan, T. H.; Li, C. J.; Lee, M. C.; Wei, Z. Y. *Can. J. Chem.* **1994**, *72*, 1181-1192.
(8) Whitesides, G. M.; Schmid, W.; Gordon, D. M.; Kim, E. *J. Org. Chem.* **1993**, *58*, 5500-5507.
(9) Whitesides, G. M.; Gordon, D. M. *J. Org. Chem.* **1993**, *58*, 7937-7938.
(10) Whitesides, G. M.; Gordon, D. M.; Härter, R.; Gao, J. *J. Org. Chem.* **1994**, *59*, 3714-3715.
(11) Schmid, W.; Binder, W. H.; Prenner, R. H. *Tetrahedron* **1994**, *50*, 749-758.
(12) Einhorn, C.; Einhorn, J.; Luche, J. L. *Synthesis* **1989**, 787.
(13) Gao, X.; Huang, B.; Wu, S. *Synth. Commun.* **1990**, *20*, 1279.
(14) Luche, J. L.; Pétrier, C.; Barboza, J. C. D. S. *J. Org. Chem.* **1988**, *53*, 1212-1218.
(15) Wade, K.; Barnister, A . J. In *Comprehensive Inorganic Chemistry*, ed. by Bailar, J. C.; Emeleus, H. J.; Nyholm, R.; Trotman-Dickenson, A. F., Eds., Pergamon Press: Oxford, 1973 Vol. 1, pp 993-1085.
(16) *The Merck Index*, Budavari, S.; O'Neil, M. J.; Smith, A.; Heckelman, P. E., Eds., 11th Ed., 1989.
(17) Schmid, W.; Binder, W. H.; Prenner, R. H. *Liebigs Ann. Chem.* **1994**, 73-78.
(18) Chan, T. H.; Li, C. J. *Tetrahedron Lett.* **1991**, *32*, 7017-7020.
(19) Wakefield, B. J. *Comprehensive Organometallic Chemistry*; Wilkinson, G., Ed.; Pergamon Press: Oxford, 1982; Vol.7 pp 63-65.
(20) Butsugan, Y.; Ito, H.; Araki, S. *J. Org. Chem.* **1988**, *53*, 1831-1833.
(21) Butsugan, Y.; Katsumura, N.; Araki, S. *J. Organomet. Chem.* **1991**, *415*, 7-24.
(22) Butsugan, Y.; Mori, A.; Yamada, M.; Shimizu, K.; Imai, A.; Araki, S. *J. Org. Chem.* **1995**, *60*, 1841-1847.
(23) Mikhailov, B. M. *Pure Appl. Chem.* **1974**, *39*, 505-523.
(24) Miller, R. B.; Reichenbach, T. *Synth. Commun.* **1976**, *6*, 319-323.
(25) Butsugan, Y.; Kato, M.; Usui, H.; Araki, S. *J. Am. Chem. Soc.* **1996**, *118*, 4699-4700.
(26) Normant, J. F.; Alexakis, A. *Synthesis* **1981**, 841-870.
(27) Butsugan, Y.; Kawai, M.; Yamamura, H.; Shimizu, H.; Hirashita, T.; Araki, S. *Tetrahedron Lett.* **1996**, *37*, 8417-8420.
(28) Loh, T. P.; Ho, D. S. C.; Xu, K. C.; Sim, K. Y. *Tetrahedron Lett.* **1997**, *38*, 865-868.
(29) Paquette, L. A.; Lobben, P. C. *J. Am. Chem. Soc.* **1996**, *118*, 1917-1930.
(30) Chan, T. H.; Isaac, M. B. *Tetrahedron Lett.* **1995**, *36*, 8957-8960.
(31) Paquette, L. A.; Lobben, P. C. *J. Am. Chem. Soc.* **1996**, *118*, 1931-1937.
(32) Chan, T. H.; Li, C.-J. *J. Chem. Soc., Chem. Commun.* **1992**, 747-748.
(33) Chan, T. H. *J. Org. Chem.* **1995**, *60*, 4228-4232.
(34) Whitesides, G. M.; Lee, S.; Choi, S.-K. *J. Org. Chem.* **1996**, *61*, 8739-8745.
(35) Marshall, J. A.; Hinkle, K. W. *J. Org. Chem.* **1995**, *60*, 1920-1921.
(36) Marshall, J. A.; Hinkle, K. W. *J. Org. Chem.* **1996**, *61*, 105-108.
(37) Li, C. J.; Chen D. L. *Tetrahedon Lett.* **1996**, *37*, 295-298.
(38) Loh, T. P.; Li, X. R. *Tetrahedron Lett.* **1997**, *38*, 869-872.
(39) Chan, T. H.; Isaac, M. B. *Pure Appl. Chem.* **1996**, *68*, 919-924.

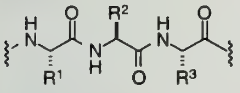
UNNATURAL BIOPOLYMERS

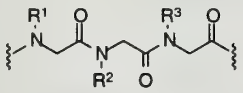
Reported by Yoonkyung Kim May 5, 1997

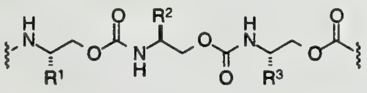
INTRODUCTION

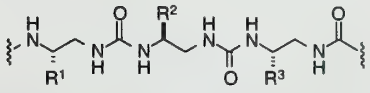
Polypeptides are attractive targets for medicinal chemists interested in the discovery of new lead compounds. However, the poor membrane permeability of peptides, and their rapid degradation and clearance have led to the development of peptidomimetics which have similar structures and biological activities but manifest increased pharmacokinetic properties resulting from metabolic stability, high selectivity (minimal side effects), high activity (low dosage), and favorable solubility characteristics.[1,2] The alterations in peptidomimetics include peptide side-chain manipulations, amino acid extensions, deletions, substitutions, and backbone modifications.

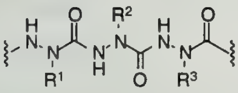
Peptides with altered backbone structures are referred as "unnatural biopolymers", or "biopolymer mimetics" and have been developed rapidly over the past five years.[3-5] Modification of the peptide framework may arise from inversion of configuration to yield retro-inverso isomers,[6] isosteric replacement of the amide group, transition state mimicry of peptide hydrolysis, and conformational modifications including restriction and extension. In the design and synthesis of new unnatural biopolymers, four factors must be considered. First, the synthesis should be straightforward and iterative. Second, monomers should be able to accomodate a variety of functional groups in their side-chains. Third, coupling reactions should be efficient, high yielding, and amenable to automation. Fourth, the linkages should be resistant to enzymatic cleavage. In this report, recent advances of six classes of unnatural biopolymers are discussed focusing on their synthetic accessibilities.

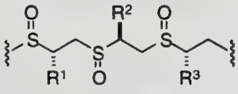

peptide

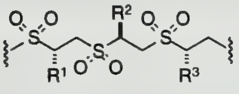

peptoid

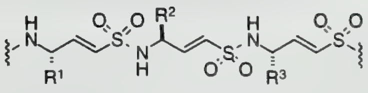

oligocarbamate


oligourea


azatide


oligosulfoxide


oligosulfone


vinylogous sulfonamido-pseudopeptide

PEPTOIDS

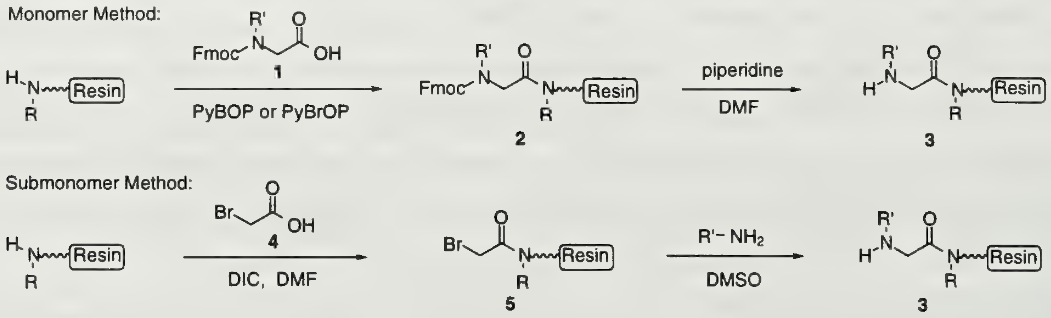
Oligomers of *N*-substituted glycines, or "peptoids", were proposed as new peptidomimetic backbones.[7] In a peptoid, side chains of the original peptide are shifted to amide nitrogen atoms, generating achiral tertiary amine groups. The advantages of the peptoids are metabolic stability, high flexibility, functional group variability in the synthesis, and the absence of chirality.

Two main approaches are known for the synthesis of peptoids (Scheme 1).[8] In monomer method,

Copyright © 1997 by Yoonkyung Kim

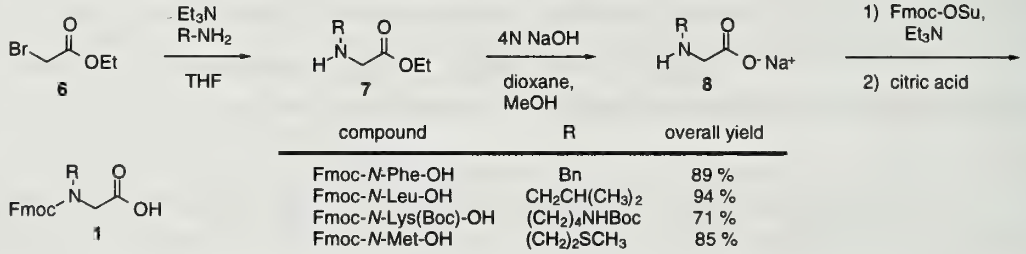
reductive amination of a side-chain protected amine with glyoxylic acid, followed by Fmoc-protection of the amino group afforded monomer **1** which was used for solid-phase chain extension.[7] A solid-phase submonomer method was developed which involves two steps per cycle.[9] The synthetic utility of the submonomer method was demonstrated by the synthesis of a 25-mer, $[(N\text{-butylglycine})_4(N\text{-(3-aminopropyl)glycine})]_5$, in 86% yield and 65% purity as determined by HPLC.

Scheme 1



Recently, an improved monomer method was reported based upon the above two strategies (Scheme 2).[10] In this approach, large excess of reagents needed in the submonomer method are avoided and reaction completion can be monitored through quantitative Fmoc analysis. In addition, intermediates **7** and **8** can be easily purified by simple column chromatography and crystallization in high overall yields.

Scheme 2



compound	R	overall yield
Fmoc-*N*-Phe-OH	Bn	89 %
Fmoc-*N*-Leu-OH	$CH_2CH(CH_3)_2$	94 %
Fmoc-*N*-Lys(Boc)-OH	$(CH_2)_4NHBoc$	71 %
Fmoc-*N*-Met-OH	$(CH_2)_2SCH_3$	85 %

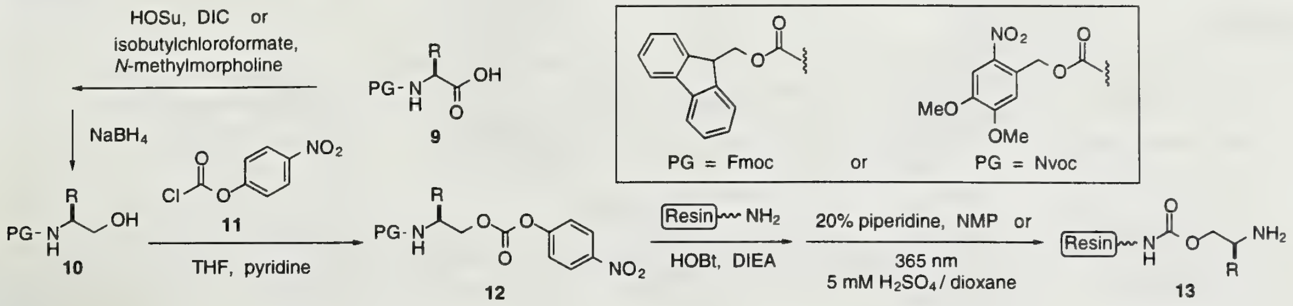
Ramachandran plots of modeled peptoids suggested a greater diversity of conformational states for peptoids compared to peptides. Binding studies of peptoids with α-amylase, hepatitis A virus proteinase, and HIV transactivator-responsive element RNA indicated that translation of the sequence of a peptide ligand into a peptoid mimic requires a retropeptoid sequence. By screening a 5000-membered peptoid library, ligands with K_i values of 5-6 nM for 7-transmembrane G-protein-coupled receptors were discovered.[11] To increase the diversity, post-modification of peptoid side-chains through [3+2] cycloaddition of nitrile oxides was carried out in solid-phase with high purity (> 80%).[12]

OLIGOCARBAMATES

A new class of backbone modified peptides was designed by replacing the amide linkages with carbamates.[3,5] This class of polymer is expected to display increased lipophilicity, altered hydrogen-bonding properties, metabolic stability, and high conformational flexibility compared to peptides.

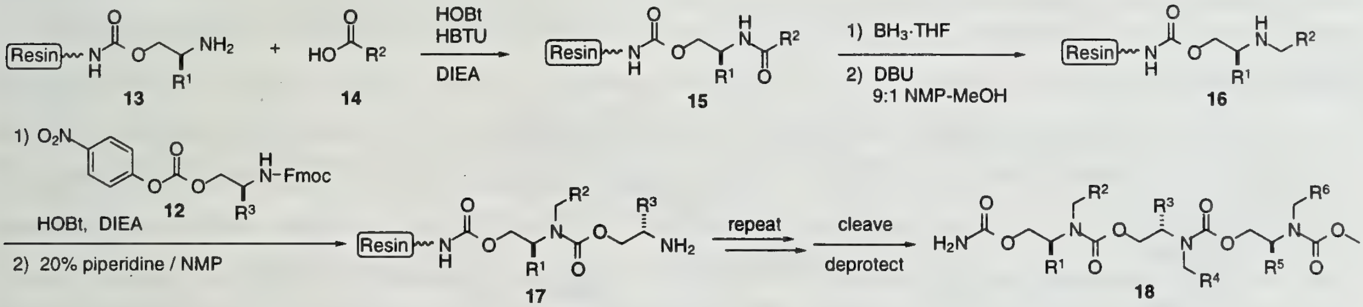
The synthesis of oligocarbamates in the solid-phase was accomplished using an activated, *N*-protected aminocarbonate monomer **12** which was prepared from a *N*-protected amino alcohol **10** (Scheme 3). The amino group was protected by either Fmoc or Nvoc (nitroveratryloxycarbonyl) groups which were deprotected by base and light, respectively.

Scheme 3



Further conformational restriction on the oligocarbamate framework was achieved by selective alkylation of the main-chain carbamate nitrogen atoms, yielding *N*-alkylcarbamate oligomers (Scheme 4).[13] This modification provides increased density of side-chains, removes main-chain hydrogen-bond donors, and reduces conformational freedom of the backbone. The synthetic route is compatible with a variety of side-chain residues (amino, guanidine, hydroxyl, etc.) and the oligomer product could be isolated in reasonably high yield (70-90% for pentamers) and high purity.

Scheme 4



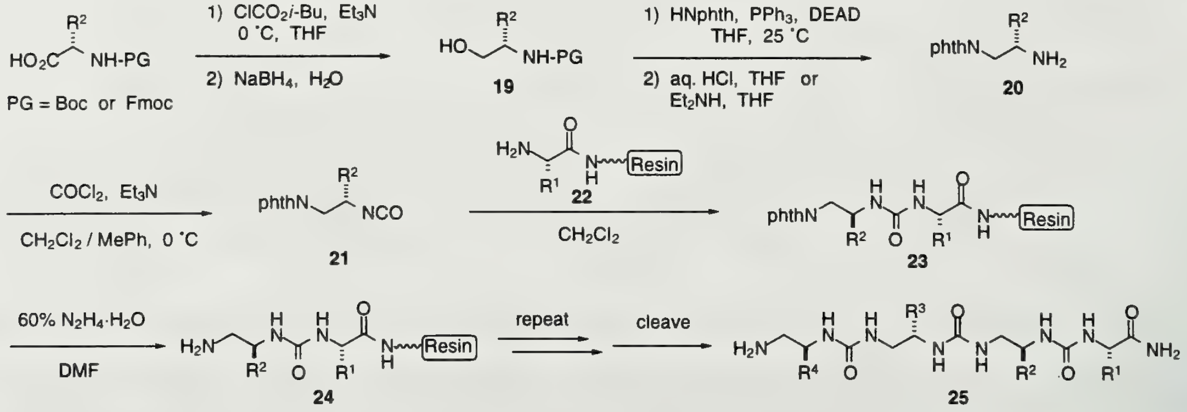
A combinatorial library of 256 oligocarbamates was constructed using Nvoc-protected monomers.[3] Screening the BSA conjugates of high afiinity ligands to monoclonal antibody 20D6.3 gave IC_{50} values of 60-180 nM. Comparison of water-octanol partition coefficients for a peptide and its corresponding oligocarbamate indicated that the oligocarbamates are more hydrophobic. Enzymatic degradation studies of oligocarbamates and peptides indicated a higher metabolic stability for the oligocarbamates.

OLIGOUREAS

An intriguing isostere of a peptide backbone was investigated by substituting the main-chain amides with urea linkages.[14-16] Ureas have been used as critical structural elements in enzyme inhibitors and as switching points in retro-inverso peptidomimetics.[6] Oligoureas have backbones with hydrogen-bonding groups, chiral centers, a significant degree of conformational restriction, and NH sites for the introduction of additional side-chain groups.

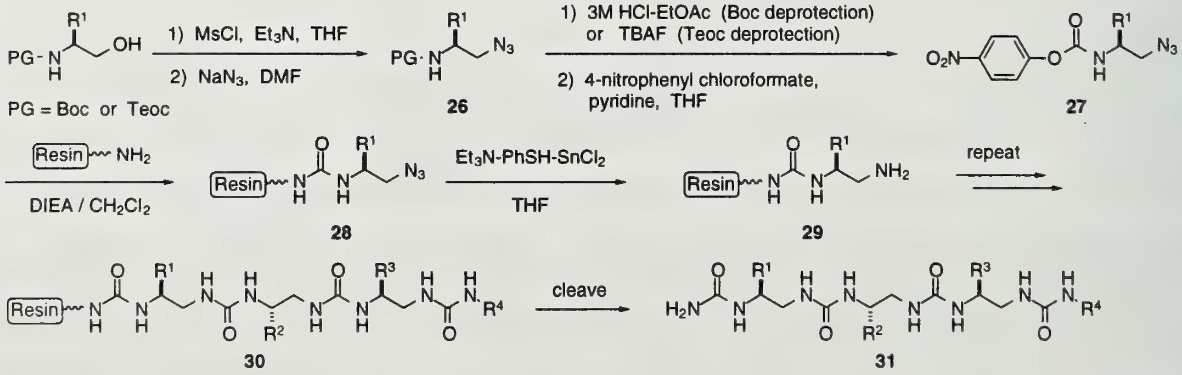
Two approaches were reported for the solid-phase oligourea synthesis. Mitsunobu displacement an *N*-protected amino alcohol **19** with phthalimide, followed by *in situ* generation of isocyanate **21**, subsequent coupling to Rink amide resin and deprotection afforded free amine **24** (Scheme 5).[14,16]

Scheme 5



Another solid-phase synthesis using activated *p*-nitrophenylcarbamate and protected amines in the form of azides was reported (Scheme 6).[15] Monomers with alkyl side-chains were prepared from *N*-Boc protected amino alcohols, while those with acid labile side-chains were synthesized from *N*-Teoc (2-(trimethylsilyl)ethoxycarbonyl) protected amino alcohols. The activated monomer **27** was reactive enough for the coupling and yet stable enough for purification by silica gel column chromatography.

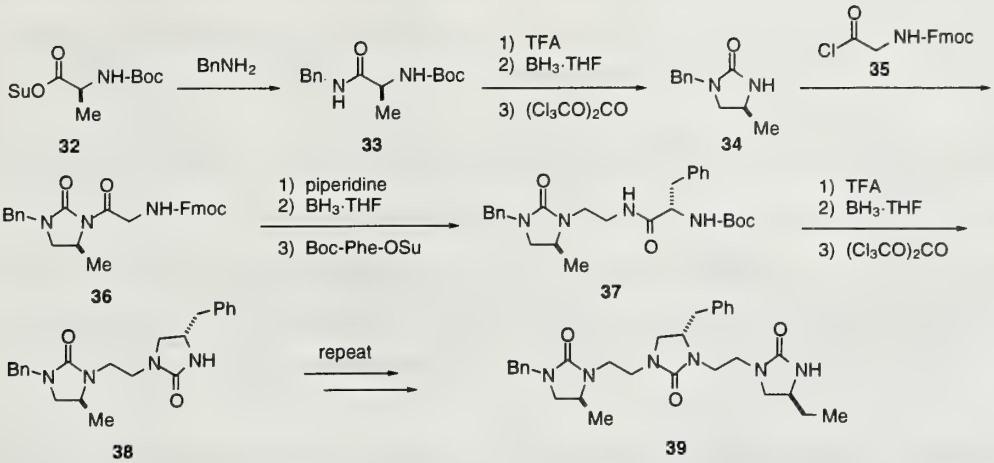
Scheme 6



Further modification of oligourea backbone was carried out by switching to a cyclic urea.[17] Cyclic ureas are found in many biologically active compounds and are expected to have greater membrane solubility and increased conformational rigidity relative to the corresponding linear urea. A trimer **39** was synthesized in an iterative approach (Scheme 7).

Aqueous buffer-octanol partition coefficient measurements indicated similar hydrophilicities for peptides and their corresponding oligoureas. A 160-membered oligourea library was screened against anti-β-endorphin antibody to give increased IC_{50} values of 25-108 μM compared to peptide analogs.

Scheme 7

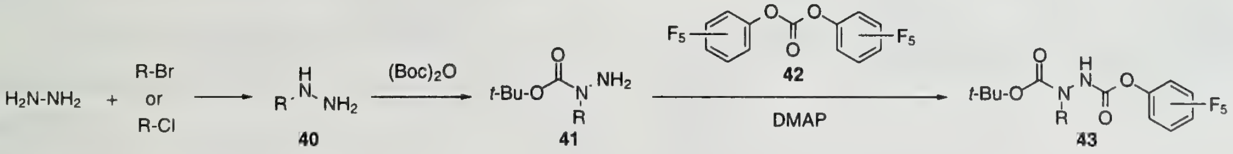


AZATIDES

A complete isosteric replacement of α-carbons by nitrogens in peptide scaffold was achieved through solution and liquid-phase synthesis.[18,19] Pioneering work in this field was done by replacing the α-carbon of a valine residue with nitrogen in bovine angiotensin II, which resulted in similar biological activity and increased metabolic stability.[20] In "azapeptides", peptides with one (or more) α-carbon(s) replaced by a nitrogen atom, the side-chains are expected to take up a position somewhere between R- and S-configuration.[21] Measurements of azapeptides have shown that the NH group attached to α-nitrogen has higher acidity than in peptides favoring stronger hydrogen-bonding. On the other hand, it was found that the protonation at physiological pH was prevented.

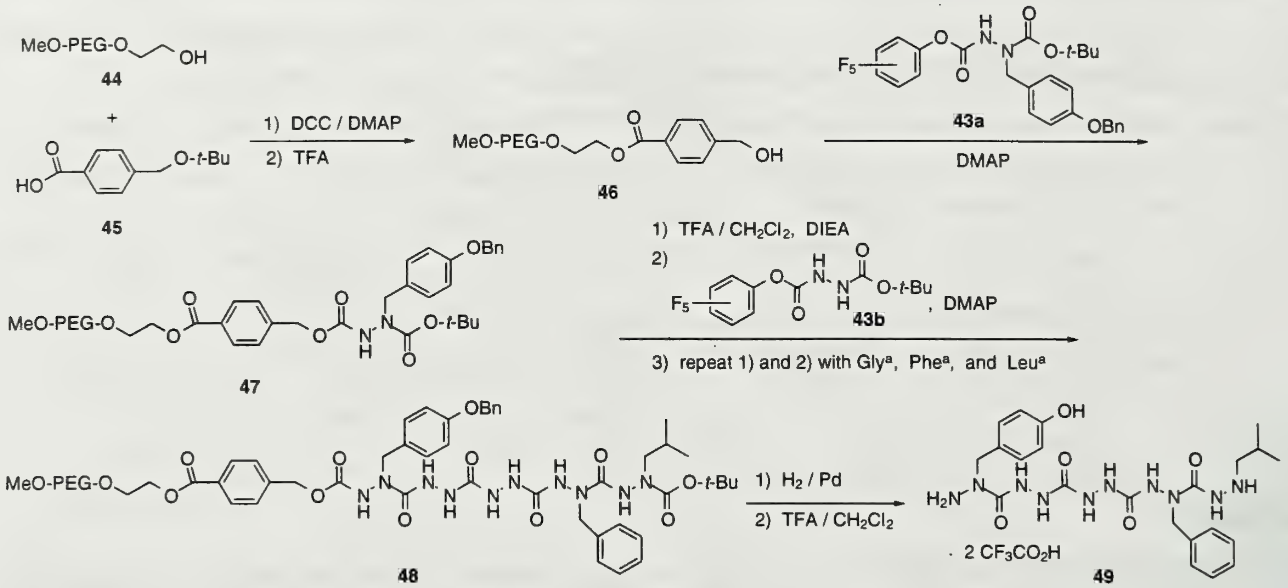
Although diverse synthetic approaches have been developed for the synthesis of azapeptides, none of these methods can be used to make a "pure azapeptide", or an "azatide". The first synthesis of an azatide pentamer began with alkylation of hydrazine followed by Boc protection to give **41**. Bis(pentafluorophenyl) carbonate **42** was chosen as the activating reagent because of the ease of preparation, strong electron-withdrawing effect with minimized steric problems, and the formation of highly crystalline products (Scheme 8).[18] However, the active species was not carbamate **43**, but rather the isocyanate which was formed *in situ* under appropriate coupling conditions. Iterative coupling and deprotection using the activated monomer **43** on MeO-PEG (poly(ethylene glycol) monomethyl ether) support through a *p*-substituted benzyl ester linker gave azatide **49** (Scheme 9).

Scheme 8



Preliminary results obtained from the binding studies with monoclonal antibody 3-E7 raised against antigen β-endorphin suggested that azatide **49** may adopt a more extended conformation within the critical glycine region.[18]

Scheme 9

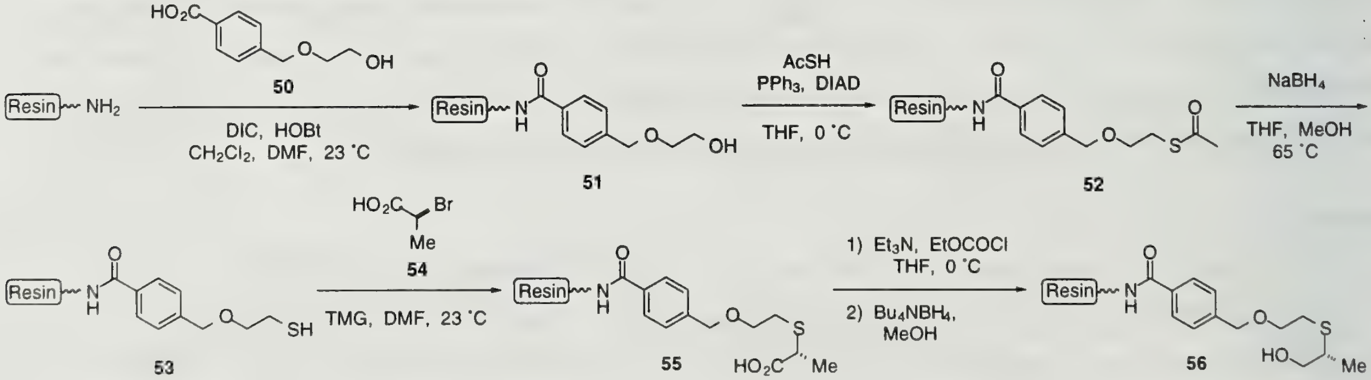


OLIGOSULFOXIDES / OLIGOSULFONES

Replacement of the peptide backbone by sulfoxide and sulfone linkages has been investigated.[5] Significant differences in backbone structure in oligosulfoxides and oligosulfones compared to peptides may give rise to unique hydrogen-bonding patterns, conformational flexibility, and biological activities.

In the synthesis of common thioether precursor **56**, 2-(4'-carboxybenzyloxy)ethanol **50** was incorporated as a chromophore to monitor the reaction efficiency (Scheme 10). One cycle of chain elongation involves five steps. Mitsunobu reaction of **51** using thioacetic acid gave thioacetate **52**, and sequential reduction and alkylation with **54** generated **55**. Reduction of **55** regenerated a terminal alcohol which can enter additional chain extension cycles. Oxidation of the oligothioether using sodium periodate[22] or tetra-*n*-butylammonium oxone[23] produced the oligosulfoxide and oligosulfone, respectively.

Scheme 10



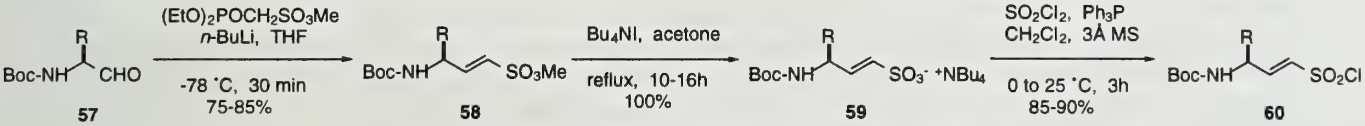
VINYLOGOUS SULFONAMIDO-PSEUDOPEPTIDES

Sulfonamides are more stable to protease degradation and possess higher flexibility compared to peptides. In addition, their stronger polarizability makes the NH group more acidic (pK_a = 10-11) and favors stronger hydrogen-bonding, which results in a more distinct preorganization of sulfonamide oligomers. Unfortunately, α-aminosulfonamides were found to be unstable and immediately decompose by fragmentation. Two preliminary synthetic approaches for β-aminosulfonamide trimers were reported.[24,25]

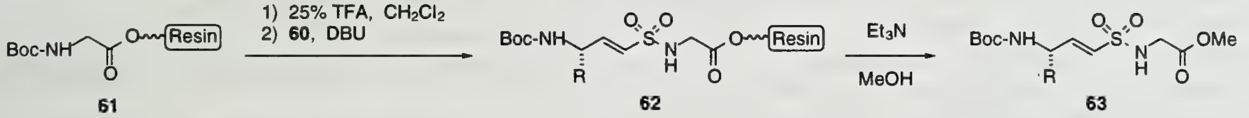
Modified peptides composed of extended amino acids that have an *E*-ethenyl unit inserted between the carbonyl carbon and C_α, vinylogous polypeptides, have been synthesized.[24,26] The conformational studies of the vinylogous polypeptides based on X-ray crystallography and ^{1}H NMR spectroscopy indicated that these frameworks can adopt novel secondary structures.[27] Through the incorporation of this additional ethenyl unit into the sulfonamide backbone structure, a vinylogous sulfonamido-pseudopeptide was designed.

A vinylogous sulfonamido-pseudopeptide was synthesized from sulfonyl chloride monomers **60** (Scheme 11). Wittig-Horner reaction of *N*-Boc amino aldehyde **57** generated the corresponding α,β-unsaturated sulfonate **58** with complete *E* stereoselectivity. Cleavage of methyl ester, followed by activation with SO_2Cl_2/PPh_3 gave sulfonyl chloride monomer **60**. Stepwise chain extension was carried out through iterative deprotection-coupling on Tenta Gel® (Scheme 12).

Scheme 11

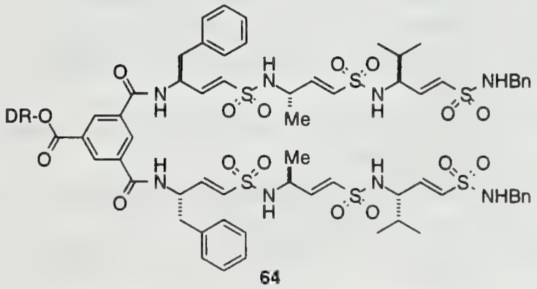


Scheme 12

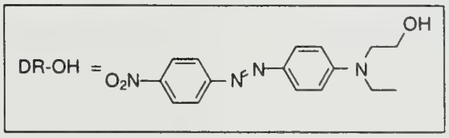


A detailed conformational study of these chiral vinylogous sulfonamido-pseudopeptides was reported by a combination of X-ray crystallography, VT ^{1}H NMR spectroscopy, FT-IR spectroscopy, NOE experiments, and computer modeling.[28] The free rotation of the C-C bond connecting the double bond with allylic stereocenter can be restricted through formation of favorable secondary structures in oligomer. Fourteen-membered ring hydrogen-bonding was found to be the most common folding pattern for oligomers.

Binding studies of tweezerlike molecular receptors based on vinylogous sulfonamido-pseudopeptides **64** with a combinatorial tripeptide library showed that these receptors bind oligopeptides with comparable binding strength (low) but different selectivity (high) to their peptide analogs.[29]



64



CONCLUSION

Unnatural biopolymers have unique properties in hydrogen-bonding, water solubility, conformational flexibility, and binding specificity resulting from their specific structural modifications of peptides. Therefore, unnatural biopolymers may afford improved frameworks for drug design as well as new folding patterns to test current notions of polypeptide structure and folding.

REFERENCES

(1) Giannis, A.; Kolter, T. *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1244.
(2) Gante, J. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1699.
(3) Cho, C. Y.; Moran, E. J.; Cherry, S. R.; Stephans, J. C.; Fodor, S. P. A.; Adams, C. L.; Sundaram, A.; Jacobs, J. W.; Schultz, P. G. *Science* **1993**, *261*, 1303.
(4) Liskamp, R. M. J. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 633.
(5) Moran, E. J.; Wilson, T. E.; Cho, C. Y.; Cherry, S. R.; Schultz, P. G. *Biopolymers* **1995**, *37*, 213.
(6) Chorev, M.; Goodman, M. *Acc. Chem. Res.* **1993**, *26*, 266.
(7) Simon, R. J.; Kania, R. S.; Zuckermann, R. N.; Huebner, V. D.; Jewell, D. A.; Banville, S.; Ng, S.; Wang, L.; Rosenberg, S.; Marlowe, C. K.; Spellmeyer, D. C.; Tan, R.; Frankel, A. D.; Santi, D. V.; Cohen, F. E.; Bartlett, P. A. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 9367.
(8) Kessler, H. *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 543.
(9) Zuckermann, R. N.; Kerr, J. M.; Kent, S. B. H.; Moos, W. H. *J. Am. Chem. Soc.* **1992**, *114*, 10646.
(10) Kruijtzer, J. A. W.; Liskamp, R. M. J. *Tetrahedron Lett.* **1995**, *36*, 6969.
(11) Zuckermann, R. N.; Martin, E. J.; Spellmeyer, D. C.; Stauber, G. B.; Shoemaker, K. R.; Kerr, J. M.; Figliozzi, G. M.; Goff, D. A.; Siani, M. A.; Simon, R. J.; Banville, S. C.; Brown, E. G.; Wang, L.; Richter, L. S.; Moos, W. H. *J. Med. Chem.* **1994**, *37*, 2678.
(12) Pei, Y.; Moos, W. H. *Tetrahedron Lett.* **1994**, *35*, 5825.
(13) Paikoff, S. J.; Wilson, T. E.; Cho, C. Y.; Schultz, P. G. *Tetrahedron Lett.* **1996**, *37*, 5653.
(14) Burgess, K.; Linthicum, S.; Shin, H. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 907.
(15) Kim, J.-M.; Bi, Y.; Paikoff, S. J.; Schultz, P. G. *Tetrahedron Lett.* **1996**, *37*, 5305.
(16) Burgess, K.; Ibarzo, J.; Linthicum, D. S.; Russell, D. H.; Shin, H.; Shitangkoon, A.; Totani, R.; Zhang, A. J. *J. Am. Chem. Soc.* **1997**, *119*, 1556.
(17) Kim, J.-M.; Wilson, T. E.; Norman T. C.; Schultz, P. G. *Tetrahedron Lett.* **1996**, *37*, 5309.
(18) Han, H.; Janda, K. D. *J. Am. Chem. Soc.* **1996**, *118*, 2539.
(19) Han, H.; Wolfe, M. M.; Brenner, S.; Janda, K. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 6419.
(20) Hess, H.-J.; Moreland, W. T.; Laubach, G. D. *J. Am. Chem. Soc.* **1963**, *85*, 4040.
(21) Gante, J. *Synthesis* **1989**, 405.
(22) Hartzell, G. E.; Paige, J. N. *J. Am. Chem. Soc.* **1966**, *88*, 2616.
(23) Trost, B. M.; Braslau, R. *J. Org. Chem.* **1988**, *53*, 532.
(24) Gennari, C.; Salom, B.; Potenza, D.; Williams, A. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 2067.
(25) Moree, W. J.; van der Marel, G. A.; Liskamp, R. J. *J. Org. Chem.* **1995**, *60*, 5157.
(26) Gennari, C.; Nestler, H. P.; Salom, B.; Still, W. C. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1763.
(27) Hagihara, M.; Anthony, N. J.; Stout, T. J.; Clardy, J.; Schreiber, S. L. *J. Am. Chem. Soc.* **1992**, *114*, 6568.
(28) Gennari, C.; Salom, B.; Potenza, D.; Longari, C.; Fioravanzo, E.; Carugo, O.; Sardone, N. *Chem. Eur. J.* **1996**, *2*, 644.
(29) Gennari, C.; Nestler, H. P.; Salom, B.; Still, W. C. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1765.

30 02/00
9595 143 ICI

UNIVERSITY OF ILLINOIS-URBANA
3 0112 002395520

UNIVERSITY OF ILLINOIS-URBANA
3 0112 037724660